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SEPARATION AND IDENTIFICATION OF FOOD-COLORING SUBSTANCES.

By W. E. MATHEWSON, *Assistant Chemist.*

CONTENTS.

	Page.		Page.
Introduction.....	1	Separation and purification of coloring substances.....	8
General statements concerning reagents used in color analysis.....	2	Identification of coloring substances.....	35
Preliminary treatment of food products.....	4		

INTRODUCTION.

The scheme of analysis of dyes described in this bulletin embrace about 130 chemical individuals. This number comprises practically all those coal-tar colors (except a few entirely obsolete nitro dyes) which have been mentioned in the literature as having been found in food products, and those mentioned as being suitable for the coloring of foods. A number of dyes which are typical representatives of certain classes and which exemplify in a general way the analytical properties of certain groups, also have been included. No process for the analysis of dyes can be made so complete as to take into account all possible colors and combinations of colors; nevertheless the analyst should be prepared for as many as possible of the cases arising. It is especially difficult to take the newer colors into consideration, but, fortunately, at the present time these are very little used in food products.

The scheme of separation described is designed to meet actual conditions, one of which is the relatively more frequent occurrence of the eight colors the use of which in food is permitted by the United States Department of Agriculture¹—Amaranth, Ponceau 3 R, Ery-

¹ Food inspection decisions Nos. 76 and 164.

NOTE.—This bulletin will be of interest chiefly to chemists engaged in food analysis.

throsin, Orange I, Naphthol yellow S, Tartrazin, Light green S. F. yellowish, and Indigo disulfoacid—and among nonpermitted dyes of the oxy-monazo colors. An entirely different scheme might be preferable if, for instance, the difficultly soluble benzidin dyes were the most common colors.

The method for the separation of colors described in this bulletin is based mainly upon the employment of immiscible solvents. By this means most mixtures of the commonly occurring coal-tar dyes may be separated with relative ease. In dealing with the natural coloring substances the analyst is hampered by the lack of any exact knowledge concerning many of them, by the difficulty of obtaining pure preparations free from accompanying colored substances, by the lack of good methods for their quantitative estimation, and by the fact that little is known regarding the stability of many of them with the common reagents. Although the most important natural colors have been included in the tables, little attempt has been made to indicate means of separation other than by the methods more suitable for synthetic dyes.

Of the colors used in developing the methods described in this paper about 40 of the commonest were synthesized and purified in the laboratory. The physical and chemical properties of the others indicated their identity and proved that the samples were of sufficient purity for practical purposes.¹

GENERAL STATEMENTS CONCERNING REAGENTS USED IN COLOR ANALYSIS.

The examination of food-coloring matters requires the frequent employment of some reagents that are not so often used in other kinds of chemical work. In addition to the ordinary acids and alkalies it is convenient for many purposes to have solutions of hydrochloric acid and of sodium hydroxid of accurately known strengths. Five-normal and tenth-normal hydrochloric acid and tenth-normal sodium hydroxid may be kept in stock bottles provided with attached burettes and guard tubes. Of the dilute solutions, eighth-normal is the most convenient concentration for use in separations, but tenth-normal solutions serve the purpose well and are to be preferred because of their greater suitability for titrations. A standard solution of five-normal sodium hydroxid is also needed and a portion may be kept in a 500-cc bottle, through the rubber stopper of which passes a graduated 10-cc pipette which is capped or closed above when not in use.

¹ The coal-tar dyes have been designated in this bulletin by the numbers given in the tables in *A Systematic Survey of the Organic Coloring Matters*; by A. G. Green. Founded on the German of Drs. G. Schultz and P. Julius, second edition, London and New York, 1904. On page 56 have been given the corresponding numbers used by G. Schultz, *Farbstofftabellen*, Berlin, 1911-1914, and by Mulliken, *A Method for the Identification of Pure Organic Compounds*, vol. 3, New York, 1910.

The solutions named below are usually dropped from a pipette into the solutions under examination. For convenience they should be kept in bottles provided with glass caps ground on an enlargement of the neck. With this form of bottle a short graduated pipette (or piece of tubing) can be kept in the bottle under the cap. The solutions are:

Bromin water, 1 or 2 per cent. One per cent solution is about fourth-normal as oxidizing agent.

Hydrazin sulphate solution, 3.2 per cent (approximately normal as a reducing agent when oxidized to water and free nitrogen).

Sodium nitrite, 7 grams per 100 cc (approximately molecular normal).

Alpha-naphthol solution, from 10 to 20 per cent in alcohol. This solution is used in very small quantities and should be kept in a bottle of the same form as used for the other reagents but of smaller size. The solution becomes dark colored and unfit for use after standing some weeks.

Sodium hydrosulphite solution ($\text{Na}_2\text{S}_2\text{O}_4$, "Blankite"). This solution is unstable and is best prepared as needed. The writer prefers to use the powdered solid kept in a small bottle, in the cork of which is fixed a strip of sheet metal to serve as a spatula. With this arrangement the salt, which is very soluble, may be dropped, a few particles at a time, into the solution to be tested.

Potassium persulphate ($\text{K}_2\text{S}_2\text{O}_8$). The same observations apply here as under "Sodium hydrosulphite solution."

The following reagents should preferably be kept in some form of small dropping bottle: Concentrated hydrochloric acid, concentrated sulphuric acid, dilute hydrochloric acid (10 per cent), sodium hydroxid (10 per cent), ammonium hydroxid (density about 0.95), ferric chlorid (10 per cent), and stannous chlorid (40 grams in 100 cc concentrated hydrochloric acid).

Other reagents needed are: Solutions of alum, barium chlorid (or, better, barium acetate), uranium acetate or uranium sodium acetate, and sodium acetate. It is convenient to have the last-named solution approximately normal, 16 grams per 100 cc. A strong solution of lead acetate (normal, not the basic salt, 30 per cent) is used in considerable amount; and a rather carefully made 10 per cent sodium carbonate solution (double normal) is useful for separations of Orange I and for coupling reactions. Common salt solution made from C. P. sodium chlorid is useful for many separations. Two hundred and fifty grams per liter is a convenient concentration.

The following solvents are most frequently used:

Alcohol.

Amyl alcohol. This should be a good grade of "pyridin-free."

Gasoline or petroleum ether, "low boiling point," of density about 0.65. Commercial pentane, though expensive, may be preferred

occasionally as a more homogeneous product of similar properties. The most objectionable of the impurities of gasoline may be removed by washing it a few times with concentrated sulphuric acid.

Ether. The alcohol in the commercial product may be removed by washing.

Dichlorhydrin. Most commercial C. P. products contain free acid. This is seldom harmful, but a freshly washed preparation may be preferred occasionally.

Ethyl acetate. Apparently this is usually impure and before use should be washed with water to remove alcohol, etc.

Amyl acetate. Amyl acetate is suited to the same uses as ethyl acetate. It is, however, a less active solvent for many dyes, partly, no doubt, because it dissolves less water. Commercial C. P. preparations show great variability, apparently from the presence of large amounts of impurities and not merely from difference in the proportion of isomers present. The impurities, especially amyl alcohol, are much more difficult to remove than those likely to be found in commercial ethyl acetate, so that the latter solvent has been used for the work described in this bulletin, notwithstanding its solubility and ease of saponification.

Anilin. Unless recently distilled, anilin is usually strongly colored; but the coloring matters present differ widely in solubility from the sulphonated dyes for whose separation it is most frequently employed. The colored impurities do not interfere further than by inconveniently masking the course of the fractionation.

Phenol. This must be colorless and should be free from mineral acid.

Carbon tetrachlorid.

Methyl alcohol.

PRELIMINARY TREATMENT OF FOOD PRODUCTS.

It is usually necessary to begin examination of the color or colored material by treatment with some solvent that will bring the coloring matter into solution.

Commercial food colors if soluble are dissolved directly in water, care being taken that the solution, to be examined according to the scheme described on pages 9 to 20, inclusive, be not made too concentrated. Solutions of suitable concentration contain from 0.05 to 0.01 per cent actual coloring matter. If the coloring matter is a powder, blowing some of the substance from the tip of a spatula over a sheet of moistened filter paper,¹ or over the surface of concentrated sulphuric acid contained in a flat porcelain dish, ordinarily will show if it is a mixture made from dry colors.

¹ O. N. Witt, Z. Anal. Chem. 26 (1887), 100.

Candies, sirups, and other sugar products may be taken up directly with hot water.

Wines, liquors, and other alcoholic beverages may be first diluted and warmed on the water bath to remove alcohol. However, for the subsequent treatment with immiscible solvents it is not usually necessary that the alcohol be driven off as it is sufficient to dilute the sample to reduce the alcohol content to 10 per cent or less. This very often is preferable to heating the sample; but it must be borne in mind that the distribution of the coloring matters will be more or less modified by the alcohol.

Solid materials, such as fruits, flesh foods, etc., may be extracted first with 80 per cent alcohol, containing a very little acetic acid, to remove basic dyes, cochineal, etc., sensitive to alkalis. The pulp separated from the acid alcohol solution may then be digested with dilute alcohol of from 65 to 80 per cent strength, containing from 3 to 5 per cent of ammonia. If both extracts are colored it is easiest not to work them up separately, but to boil off the alcohol and ammonia from the second portion, and the alcohol from the first, and then combine them. This procedure is quite general in its applicability. However, with products colored with metallic lakes, such as those of the flavone and flavonol dyestuffs, the treatment with strong hydrochloric acid and amyl alcohol, suggested below, is perhaps more satisfactory. Lake pigments in many cases also can be washed off the surface of the food material, since they are most often used as facings. The washings are allowed to settle or are whirled in a centrifuge, and a portion of the sediment containing the lake is treated for identification of the color. Most lakes are decomposed, at least to a large extent, by hydrochloric acid and amyl alcohol.

From many solid products such as jams and meat pulp the common coloring matters, including the permitted dyes, can be extracted directly by adding concentrated hydrochloric acid and shaking thoroughly with amyl alcohol. The subsequent work is shortened and, with strongly colored materials especially, the plan is often quite satisfactory.

Wheat and rye products offer some difficulty in the extraction with dilute alcohol because of the solubility of the plant proteins, gliadin and hordein. In the case of macaroni, spaghetti, etc., boil the ammoniacal alcoholic extract containing the coloring matter until most but not quite all of the alcohol is removed. If the hot residue is of a semisolid consistency, it is best to add a little alcohol. It is then treated with about one-half of its volume of concentrated hydrochloric acid and is poured into a large separatory funnel. Amyl alcohol equal to about two-thirds of the original volume of the solution is added and sufficient salt solution to make the mixture separate well.

The amyl alcohol containing the color is washed a few times with a salt solution containing hydrochloric acid to remove the protein; one separation with the centrifuge usually being desirable to free the solvent from the coagulum. The further treatment of the amyl alcohol solution is the same as that described under the heading Separation and Purification of Coloring Substances, page 8.

Dissolved coloring matters are generally separated from fats and oils by saponifying the fat or oil with alcoholic potash and extracting the coloring matter from the soap with gasoline or ether.¹ The manipulation of this process is not very convenient and, of course, all unsaponifiable matter remains with the color. It may be combined with advantage in many cases with one of the extraction methods with an immiscible solvent described below. A number of extraction methods are in use and probably each possesses advantages for certain colors.² Some dyes, as Anilin Yellow, may be extracted from oils conveniently with 90 per cent alcohol.³ The method of Cornelison⁴ (extracting the coloring matter with glacial acetic acid or with the same solvent containing a little added hydrochloric acid or water) will serve for the extraction of almost all the common oil-soluble dyes. Much oil dissolves in the acetic acid and a systematic fractionation is necessary; the different portions of extract being washed successively in several funnels containing a little gasoline.

The writer prefers the following procedure, which, though somewhat inconvenient, is quite generally applicable. It does not give a color entirely free from cholesterol and similar compounds. About 30 cc of the oil are diluted with about 120 cc of low-boiling gasoline, and this mixture is shaken out with several portions of a mixture of 90 parts of phenol with 10 of water. The volume of the first portion of solvent may be about 45 cc, the others 30 cc each. The phenol extract is washed in a separatory funnel with 2 or 3 portions of gasoline, then treated with sufficient cool, strong potassium or sodium hydroxid solution to dissolve the phenol. The dye is removed by shaking out the liquid with from 50 to 100 cc of ether. The ether is first washed a few times with caustic alkali solution to remove all phenol and finally with water. It may then be evaporated or treated further as indicated on pages 7 and 32-33.

The Sudan dyes are readily extracted by a mixture of 80 parts phosphoric acid (85 per cent, density about 1.70) and 20 parts concentrated sulphuric acid. The oil containing the dyes should be

¹ See Gruenhut, Chem. Zentr. 69 (1898) II, 943.

² See Berry, U. S. Dept. Agr., Bur. Chem. Circ. No. 25; Doolittle, U. S. Dept. Agr., Bur. Chem. Bul. No. 65, p. 152.

³ For the extraction and identification of Auramin (No. 425) when present in oils, see Frehse, Ann. fals 3 (1910), 293.

⁴ J. Am. Chem. Soc. 30 (1908), 1478.

diluted with a few volumes of gasoline. Shaking out one or two times will usually be sufficient, but the method is not applicable to colors such as tolueneazo- β -naphthylamin, which are sensitive to strong acids. The alkali salts of Sudan G and of the coloring matter of annatto are readily soluble in water; hence these dyes are most easily removed by shaking out with dilute sodium or potassium hydroxid solution.

The extraction and separation of the dissolved coloring matters may be carried out together as follows: The oil or melted fat is diluted with gasoline and shaken out first with 2 per cent (half-normal) sodium hydroxid solution to remove annatto, Sudan G, and colors of similar solubility. The mixture is then washed several times, if necessary, with hydrochloric acid of from four to six normal strength, which will take out the aminoazo derivatives, such as Butter Yellow and aminoazotoluene. Benzeneazo- β -naphthylamin and tolueneazo- β -naphthylamin are extracted rather slowly by this treatment, the dyes apparently suffering rearrangement from the hydrazo-imin form into the true azo form before going into solution in the acid. Since the toluene derivative especially is rather rapidly decomposed by hydrochloric acid, the extracts should not be allowed to stand, but should be neutralized immediately. The Sudans and similar colors not extracted from the mixture by alkali or acid should be separated by one of the general procedures described on page 6, most conveniently with the phosphoric acid mixture. If the phosphoric acid solution, after washing once or twice with gasoline, be diluted and partially neutralized, the coloring matter in quite pure condition can be obtained by extraction with ether or gasoline.

Glycerol,¹ sodium salicylate² solution, and a mixture of these two have been recommended for the extraction of colors from some food products.

Microscopic examination of colored products usually gives useful information. This is especially true in certain cases for chemical tests under the microscope.³

Certain coloring matters can not be brought into solution by the methods outlined. Such substances are the organic pigments Indanthrene (No. 569) and unsulphonated indigo (No. 690), which are insoluble in all ordinary solvents and must be identified by their general properties. Lampblack and similar forms of carbon are characterized by insolubility in acids, alkalis, or hot dichlorhydrin, and by their complete combustibility. Ultramarine is stable toward alkalis but is very readily decolorized with acids with evolution of hydrogen sulphid, which may be detected with lead acetate paper.

¹ Klinger and Bujard, *Z. Angew. Chem.* (1891), 515.

² E. Spaeth, *Z. Nahr. Genussm.* **18** (1909), 587.

³ Winton, A. L., *The Microscopy of Vegetable Foods*. New York, 1916.

Most other pigments not lakes are compounds with heavy metals and a suitable examination according to the ordinary methods of inorganic analysis will indicate the nature of the pigment.¹

SEPARATION AND PURIFICATION OF COLORING SUBSTANCES.

PRELIMINARY TREATMENT.

By suitable preliminary treatment the coloring matter should be obtained in aqueous or dilute alcoholic solution nearly free from acids, alkalis, or large quantities of salt. (Concerning the oil-soluble dyes see pages 6 and 7.) The alcohol content of the solution should not exceed 10 per cent. Usually it is better to remove excessive alcohol (by evaporation) than to add water; but if the liquid contains so much sugar as to be sirupy it should be diluted. If the evaporation causes a separation of coloring substance, the sediment should not be removed before the treatment with immiscible solvents. When the color has been extracted directly from solid products by acid amyl alcohol, this may be shaken out with salt solution, dilute hydrochloric acid, or water, as directed for the corresponding solution obtained in the first step of the procedure described on pages 11, 17, and 18.

Since the coloring substances of flowers and fruits are, generally speaking, rather unstable, especially in the presence of alkalis, it is well to divide the solution containing the colors into two portions, one portion to be examined for the natural colors, the other for coal-tar dyes.

TREATMENT OF SOLUTION RESERVED FOR TESTING FOR COAL-TAR DYES.

If coal-tar dyes are not known to be present, a preliminary test may be made by warming a small piece of wool, such as nun's-veiling, or some white woolen yarn with some of the solution; first neutral, then, if no dyeing takes place, made acid with a few drops of hydrochloric acid.² If the wool is dyed in either case, the main portion of the solution reserved for dyes is treated as indicated on page 9.

Acid Yellow (sulphonated aminoazobenzene, No. 8) is sometimes more easily separated from mixtures by dyeing on wool than by the use of solvents; hence if the test wool is dyed yellow it may be stripped with dilute ammonia and this solution tested for Acid Yellow by diazotization, etc., as described on page 51.

In the presence of very large amounts of natural coloring matter, it may be advisable occasionally to make the dyeing test with a comparatively large portion of the solution, stripping and redyeing

¹ For pigments in tea compare Read, U. S. Treasury Decision No. 32322; Knight, *J. Ind. Eng. Chem.* **6** (1914), 909.

² See Strohmer, *Z. Anal. Chem.* **24** (1885), 625. Arata, *Z. Anal. Chem.* **28** (1889), 639. Winton, *Conn. Agr. Exp. Sta. Rpt.* **2** (1889), 131. Sostegni and Carpentieri, *Z. Anal. Chem.* **35** (1896), 397. Tolman, *Jour. Amer. Chem. Soc.* **27** (1905), 25.

once or twice if necessary and making a further examination of the color substance obtained from the dyed wool. This procedure is especially advantageous when dealing with cacao products, since such products give extracts containing much natural color similar in tint to the dyes likely to be present, and special care is necessary to avoid overlooking the dyes.

It is desirable to obtain as much information as possible from the dyeing test, since the separation with immiscible solvents can not well be followed with the eye when dealing with dyes of the same shade or with the natural coloring matters, which usually consist of mixtures of substances of similar tint but different solubility.

Attention must be called to the fact that a few rather common dyes are so unstable as to be very easily overlooked when making the dyeing test; for instance, Auramin (No. 425), which is largely used at the present time in European countries for food coloring, is readily decomposed both by acids and alkalies. Naphthol green B also is easily decomposed by acids and not readily dyed on wool from many mixtures. Further, many dyes do not go on wool readily in the presence of certain impurities. In such cases, although getting no positive results by the dyeing test, the analyst should proceed with the separation by immiscible solvents.

GENERAL STATEMENTS REGARDING THE SEPARATION OF DYE MIXTURES.

The analyst usually knows something in regard to the coloring matters present in a dye solution before beginning the systematic analysis. The best procedure to be followed will depend on what dyes are probably present; and no set method can equal in value a table of relative solubilities by means of which the distribution constants of any given dyes may be compared. It is, of course, advantageous in many cases to make group tests with small portions of the mixture, thus avoiding unnecessary and undesirable additions of reagents to the main solution.

In carrying out the fractionations described on pages 11 to 18 any given color will, in general, appear in several washings, but where the maximum amount comes out will be evident from the solubility data; it being always remembered that these statements apply to solutions of concentration in the neighborhood of 0.01 per cent, and that at widely different dilutions some variation may be expected. The solubilities of the components of the dye mixture are not likely to be so different as to allow even a qualitative separation by a single shaking out. It is usually necessary to employ more or less systematic fractionation methods. For example, suppose a mixture is to be separated, of which it is known that one dye, when its amyl alcohol solution is shaken with an equal volume of acid of a certain concentration, distributes itself in equal amount between the two layers,

while 94.1 per cent of the other color, under the same conditions, remains in the aqueous layer. If such a mixture in water solution is brought to the given acidity and is shaken out successively with three portions of amyl alcohol, each equal to one-fourth its volume, calculation shows that if the distribution ratios remain constant there will be present in the acid solution after the third shaking twenty-seven sixty-fourths or 42 per cent of the first dye, one sixty-fourth or 1.6 per cent of the second. Conversely the first amyl alcohol portion after two washings with portions of acid of concentration similar to that of the original solution, will contain 42 per cent and 1.6 per cent of the second and first dyes, respectively. Obviously, for a practicable quantitative separation, somewhat greater differences in solubility must exist; but it is usually sufficient to separate, in fairly pure condition, a portion of each of the colors that seem to be present, in order to characterize completely the components of the mixture.¹

Emulsions occasionally cause trouble when the analyst is working with impure mixtures. These are most effectively broken by a good centrifuge. Should a solid stratum form between the two layers, it should be broken with a glass rod, the tube replaced in the centrifuge and whirled again. Heating tends to promote rapid separation, but the relative solubilities vary somewhat in hot mixtures. Strong acid solutions show much less tendency to emulsify than neutral or alkaline ones.

The final separation of mixtures of dyes of rather similar solubility will usually be made by selecting some pair of solvents in which they show a decided difference. Mixtures of dyes of practically identical solubility can, in most cases, be separated satisfactorily by chemical means or by precipitation reactions. Since the fractionation will have removed all except a few dyes belonging to a known group, suitable chemical methods may usually be chosen without difficulty.

While the scheme described is not intended to be applied to relatively concentrated solutions, in practice in the examination of colored food products, such are seldom or never obtained. The chief concern of the analyst here will be to avoid, as far as possible, the dilution of the color by the use of unnecessarily large portions of organic solvents and washing liquids. Only in working with products sold for use as coloring matters are solutions likely to be made too concentrated to be adapted to the scheme of separation.

As the common food dyes are, for the most part, salts of polybasic acids, the equilibrium conditions are obviously quite complex and concern not only the relative solubilities and dissociation constants of the free color acid, but of the various acid salts and the sodium salt as

¹ For procedure and calculation as to quantitative fractionations, no coloring matter being rejected, see *J. Ind. Eng. Chem.* 5 (1913), 26.

well. Many dyes exist in more or less associated condition in ordinary solutions. However, it is found in practice that in most cases the distribution ratios with given acidity do not vary greatly, but that fair results can be obtained on the assumption that they will remain constant.¹

Both basic and acid triphenylmethane colors tend to undergo slow intermolecular changes when treated with acids and alkalies (adjustment of equilibrium between carbinol, imid, and ammonium forms) and their complete separation by means of solvents is less simple than that of most other classes.

It will be noticed from the solubility table, pages 22-33, that amyl alcohol, amyl alcohol gasoline mixtures, and ether, although differing greatly in their power as solvents, show a sort of general correspondence in properties. They are especially suited for fractionations of such dyes as the sulphonated phenolic compounds, the distribution ratios of these changing greatly with varying hydrogen ion concentrations. Dichlorhydrin, because of its solubility and non-volatility, is not very convenient as a solvent; nevertheless, it is almost indispensable for the separation of many colors. Anilin is an excellent solvent, but usually must be completely removed from a color solution before tests are made, and will be employed only for a few separations. Both anilin and dichlorhydrin are conveniently removed from water solutions by shaking out with carbon tetrachlorid.

It may be remarked that in working with the coal-tar dyes the plan of acidifying strongly, extracting, and then washing the solvent with a more dilute acid, is in nearly all cases preferable to the practice of gradually increasing the acidity and using a number of portions of solvent. Much more solvent is required in this latter way, with a corresponding increase in the proportion of the accompanying impurities and in the difficulties caused by emulsification.

The writer usually prefers to begin the treatment with immiscible solvents by shaking out with amyl alcohol from the neutral solution after addition of some sodium chlorid. The outline which follows will indicate approximately the order in which the solvents will be chosen for a complex mixture.

PROCEDURE OF SEPARATION.

The solution of the coloring matter, as free as possible from suspended matter, is treated carefully with sodium carbonate if it contains free mineral acid or with acetic acid if it is alkaline. It should finally be neutral or very faintly acid. It should not contain the coloring matter in too great concentration, although when working with extracts of food products this latter condition is seldom encountered. Concentrations of about 0.01 per cent may be taken as most suitable in general, and only in exceptional cases would stronger solutions (0.1 per cent, for instance) be chosen by pref-

¹ See W. Reinders and C. Sely, *Zeit. für Chem. und Ind. d. Koll.* **13** (1913), 96.

erence. In regard to the presence of alcohol, see page 8. When large amounts of dissolved foreign material, such as sugar, glycerol, etc., are present, it must be remembered that the solubilities of the coloring matters will be somewhat affected.

Since almost all coloring matters are found accompanied by small amounts of similarly colored substances of different solubilities (subsidiary dyes, etc.), it should be made an invariable rule in carrying out the separation, first to follow through, to the point of identification, those coloring matters that seem to be present in largest proportion. The course to be pursued in dealing with the smaller fractions will then be more clearly indicated.

SEC. 1.—The solution containing the coloring matter is treated with enough strong sodium chlorid solution to bring the salt concentration to about 5 or 6 per cent and is then shaken out with 20 cc or more of amyl alcohol. If a considerable amount of coloring matter is taken up the extraction is repeated once or twice, the different portions of solvent being finally combined. The amyl alcohol, if colored, is washed once or twice with small portions of 5 per cent salt solution, and these washings, if they appear to contain any dye, are added to the original extracted solution. Any suspended solid matter that may separate may be considered also to belong to the aqueous solution.¹

The amyl alcohol, if colorless or freed from color by the washing, is discarded. Basic dyes and most of the acid colors of low sulphur content are absent. If the amyl alcohol is colored it is treated as directed in section 10.

SEC. 2.—The extracted salt solution is treated with about one-half its volume of concentrated hydrochloric acid and is again shaken out with amyl alcohol, exactly as described in section 1. Should coloring matter be extracted, the combined portions of the solvent are washed once with diluted acid (1:2, approximately four-normal), then reserved for treatment as stated under section 6. If the alcohol is colorless, and remains so after treatment with an excess of ammonia solution, it is discarded; and most of the strongly sulphonated azo colors are known to be absent. (When Naphthol green B is present, compare section 18.)

SEC. 3.—The extracted acid salt solution which may appear nearly colorless is treated with ammonia until slightly alkaline, then made slightly acid with acetic acid. If it is now colorless the absence of the strongly sulphonated triphenylmethane green and blue dyes is shown, and it is discarded. If it is colored, and if the shade indicates the possible presence of green or blue colors, it is shaken out with dichlorhydrin. This solvent is slightly soluble in water, but an amount should be used so that the lower layer after separation will not measure more than 20 cc. If coloring matter of bluish tint has been extracted, the mixture is again shaken out once or twice, and the combined portions of solvent washed with a little salt solution. The dichlorhydrin solution is then examined according to section 5.

SEC. 4.—The original mixture after the preceding extractions may still contain Acid Magenta, caramel, and many natural colors, especially the glucosids (anthocyan) constituting the common fruit colors. Acid Magenta may be recognized by its reactions with nitrous acid, dyeing properties, etc. It may be separated, if desired, by adding hydrochloric acid so that the acidity is above that of a fourth-normal hydrochloric solution (allowance must be made for the ammonium acetate present) and then shaking out with anilin. The anilin solution is washed with fourth-normal hydrochloric acid in salt solution of from 5 to 6 per cent strength; and the dye then removed with water, perhaps after addition of some carbon tetrachlorid. Before testing this magenta solution the dissolved anilin must be carefully removed, by making alkaline and extracting several times with carbon tetrachlorid, benzene, or other convenient solvent. Commercial Acid Magenta is a somewhat variable mixture of sulphonates and may be expected to yield considerable fractions of lower sulphonated derivatives of greater relative solubility in organic solvents.

¹ The more systematic procedure described in J. Ind. Eng. Chem. 5 (1913), 26, may be used, if preferred, for this and other similarly described extractions.

The acid yellows (No. 8 and No. 9) although chiefly extracted by amyl alcohol from the acid solution (sec. 2) always yield a large fraction in this group. When the coloration of the extracted acid salt mixture is entirely due to such products it will be orange red, becoming yellow on neutralization, and also will show the characteristic reactions of the acid yellows with nitrous acid, etc.

SEC. 5. The dichlorhydrin solution is diluted with three or four times its volume of carbon tetrachlorid and the color removed with a few small portions of water. The combined washings should be shaken out once with carbon tetrachlorid to get rid of dissolved dichlorhydrin. The aqueous solution may contain the higher sulphonated triphenylmethane colors or perhaps sulphonated indulin. These dyes, like Acid Magenta, are accompanied by large amounts of subsidiary products, and their solubilities can not be established with any definiteness. For their further differentiation compare their properties as shown in the tables.

SEC. 6. The amyl alcohol extract of the strongly acid salt solution, if colored, is washed four or five times with fourth-normal hydrochloric acid, the washings being kept separately. No. 108 and No. 692 predominate in the first washings, while the acidity is still high, because of hydrochloric acid dissolved in the amyl alcohol. No. 106, No. 107, and No. 94 come out in large proportion when the acidity of the lower layer, after the shaking, is below seven-tenths normal (usually about the third washing). Obviously a stronger acid than fourth-normal may be used at first, but it is usually better in practice to wash with this concentration and refractionate if necessary. The dyes that may be present in the acid amyl alcohol extract show a gradual transition in their distribution ratios relative to amyl alcohol (and other like solvents) and hydrochloric acid of varying concentration. Consequently the acid normalities to be chosen in working with an unknown mixture must be selected somewhat according to probabilities.

Comparison of the appearance of the different washings usually will show whether more than one color is present which is extracted by fourth-normal hydrochloric acid in considerable proportion. The amyl alcohol is reserved for the treatment described in section 7 or 8. No. 108 may be separated from Nos. 106, 107, and 94 by fractionation between two-normal hydrochloric acid and amyl alcohol. Nos. 692 and 8 can be separated from Nos. 106, 107, and 94 similarly with eight-normal sulphuric acid and a mixture of equal volumes of amyl alcohol and gasoline; although, since the acid is somewhat difficult to remove afterwards, the procedure is better adapted for separating the last-named dyes in pure condition than Nos. 692 and 8. For Nos. 106, 107, and 94, the amyl alcohol gasoline solution is washed with a little water to take out the dye. This solution is treated with one-half its volume or more of concentrated hydrochloric acid and is reextracted with amyl alcohol. This latter solution may now be washed with a few portions of hydrochloric acid of from four to six normal strength to remove sulphuric acid. The dye is finally removed with a little water and the color obtained in pure condition (for the cyanid reaction, for example) by evaporation to dryness on the steam bath. The dyes in the sulphuric acid solution are best separated by anilin (compare p. 23); but the final removal of this solvent is tedious. No. 94 must be separated from No. 106 and No. 107 by anilin and fourth-normal hydrochloric acid in 5 or 6 per cent salt solution. After the fractionation the dissolved anilin in the solutions must be carefully removed by several extractions with carbon tetrachlorid or other convenient solvent from the faintly alkaline solution.

Commercial No. 692 and No. 8 are made by direct sulphonation of coloring matters and are rather indefinite in composition. It will often be more convenient to divide the solutions of the colors of this group and to destroy different dyes in the various portions. By cautious treatment with "Blankite" ($\text{Na}_2\text{S}_2\text{O}_4$) in acid solution, subsequently shaking with air to restore the blue, No. 692 may be separated from the azo colors.

By reduction in ammoniacal solution, avoiding excess of "Blankite," No. 106 and No. 107 may be destroyed, while No. 8 is merely converted into the hydrazo compound and may be restored by shaking with air. No. 692 is destroyed by warming in acid solution containing a little urea and a drop of sodium nitrite solution, while Nos. 106, 107, and 108 are scarcely attacked. The cyanid reaction is best suited for the examination of mixtures of No. 106 and No. 107.

The dyes of this group, because of their ready solubility in water and fruit juices, are well adapted and largely employed for food coloring. Hence the application of the data given in the solubility table, etc., has been indicated rather more fully here than for the other classes.

SEC. 7. The amyl alcohol extract after being washed with fourth-normal hydrochloric acid may be similarly washed with sixteenth-normal hydrochloric acid; although unless No. 14 or No. 188 appear to be present this step usually will be omitted.

SEC. 8. The amyl alcohol is now measured, diluted with an equal volume of low-boiling-point gasoline, and washed first with fourth-normal hydrochloric acid two or three times, then similarly with sixteenth-normal hydrochloric acid, with sixty-fourth-normal hydrochloric acid, with sixty-fourth-normal acetic acid, and finally with sixty-fourth-normal sodium hydroxid. The dyes separated here include a large number of individuals and the treatment most desirable for any given mixture can best be judged after reference to the tables, pages 24 to 29. Obviously, the normalities stated are chosen somewhat arbitrarily, any two dyes contiguous in the table usually differing little from each other in solubility. When the appearance, etc., of the different fractions indicate the presence of more than one dye, the coloring matters must be obtained in pure condition by refractionation. Although the acid amyl alcohol extract, after dilution with gasoline, appears to yield all its color to the acid washings, it must nevertheless be shaken with the alkaline solution before being discarded, since a number of the weakly acid coloring matters (most of which, it is true, do not properly come in this group) are nearly colorless when dissolved in the neutral or acid organic solvent.

Naphthol yellow S, which predominates in the first strongly acid washings, is also nearly colorless in acid solutions, and a portion from these solutions must always be tested for this dye by making double normal with hydrochloric acid and shaking with washed ethyl acetate. If the separated solvent is found by treatment with alkali to have taken up a yellow dye, the remainder of the fractions containing it are treated in the same way with the acetate. Although the washings of low acidity may contain some coloring matters, the major portion of such dyes will be in the amyl alcohol extract of the neutral salt solution. It is best, therefore, to set aside the sixty-fourth-normal acetic acid and the sixty-fourth-normal sodium hydroxid washings until after the examination of the neutral salt amyl alcohol extract has been made; or these solutions may be mixed with the corresponding ones obtained by the processes outlined in sections 11 and 12 and may be worked up with them. Or, finally, the amyl alcohol gasoline mixture, after washing with sixty-fourth-normal hydrochloric acid, may be reserved and combined with the similar mixture described in section 10.

SEC. 9. For the separation by chemical means of closely similar dyes of these groups some of the more useful general methods may be indicated here.

The reaction with cyanid (page 52) may be used for the separation of R-salt derivatives (Nos. 55, 56, 65, 15) from mixtures with isomers.

Methods based on reduction and subsequent oxidation are applicable for the destruction of azo and nitro colors in presence of most other classes of colors, as indicated in the tables of Weingartner and of Rota.

By cautious reduction in sodium carbonate or ammoniacal solution oxyazo dyes tend to be attacked more rapidly than aminoazo dyes. It must be remembered.

however, that new dyes may also be formed by partial reduction in the case of polyazo or nitroazo derivatives.

The halogenated fluorescein derivatives are much more resistant to bromin in acid solution than are most other colors. They tend, however, to add bromin unless fully substituted. Most of the azo dyes are much more readily destroyed by bromin in alkaline solution than is Naphthol yellow S. Mixtures are made fourth-normal or above with sodium carbonate and are treated with dilute bromin water very cautiously until the azo dye is just destroyed or until the solution has become a clear yellow. Hydrazin sulphate is now added quickly to destroy excess of bromin; the mixture is finally acidified, and the yellow purified by extraction with an immiscible solvent. This procedure is seldom so satisfactory as the regular extraction with ethyl acetate or amyl acetate, and is not applicable in the presence of Nos. 62, 64, 65, and 188, which form intensely blue substances by this treatment.

SEC. 10. The amyl alcohol extract obtained by shaking out the original mixture after adding 5 or 6 per cent salt will contain practically all of any basic dyes present. Most of the acid dyes of low sulphur content are also almost completely extracted. The extract is measured, diluted with an equal volume of gasoline, then washed a few times with sixty-fourth-normal hydrochloric acid. The washings, if colored, are treated as directed in section 11. The extract is next shaken out with sixty-fourth-normal acetic acid, these washings being treated according to section 12. Eosins and (in general) coloring matters that are unsulphonated phenolic compounds are now removed by a few portions of sixty-fourth-normal sodium hydroxid solution, this fraction being treated according to section 13. The amyl alcohol gasoline mixture is finally washed once with very dilute acetic acid and, if still containing any significant amount of coloring matter, is evaporated to dryness on the steam bath, the residue being examined according to section 14.

SEC. 11. The washings of sixty-fourth-normal hydrochloric acid (sec. 10) are tested for basic dyes by making a small portion alkaline with sodium hydroxid, shaking with ether, then treating the ether solution, which is usually colorless, with dilute acetic acid.¹ If the latter becomes colored, indicating the presence of basic dyes, the alkaline test portion may be shaken out once or twice more to determine whether or not acid dyes are also present in this fraction. If these tests indicate the presence of both acid and basic colors, the acid colors must be removed by making the principal part of the sixty-fourth-normal hydrochloric acid extract alkaline (normal with sodium hydroxid) and extracting with ether. From the combined ether portions the basic dyes are removed by washing—first with sixty-fourth-normal acetic acid, finally with dilute hydrochloric acid. This treatment should be omitted if acid dyes are absent, since most basic colors are unstable in alkaline solutions, Auramin, especially, suffering decomposition rapidly. The basic colors may be further fractionated from amyl alcohol with dilute hydrochloric acid, from ether with very dilute alkali, etc. The separation of basic colors from alkaline solutions by immiscible solvents is rather objectionable, since such colors (according to Kehrmann, Havas, and Grandmougin²) suffer rearrangement from ortho-quinoid to para-quinoid structure. This change is attended in compounds such as Crystal Violet, containing only fully alkylated amino groups, by elimination of one of the alkyl groups. The original dye may not be obtained therefore, but, instead, the lower alkylated derivative.

The alkaline solution, after removal of the basic dyes with ether, is made about normal with hydrochloric acid and is shaken out with amyl alcohol gasoline mixture. Any coloring matter extracted here probably will be a minor portion of a dye already obtained by the procedure described under section 8, and its further fractionation will be carried out as stated in that paragraph; or the solution containing it may be combined

¹ O. N. Witt, *Z. Anal. Chem.* **26** (1887), 100. Weingärtner, *Z. Anal. Chem.* **27** (1888), 232.

² *Ber. Chem. Ges.* **46** (1913), 2131; **47** (1914), 1881.

with the similar solution obtained by the procedure outlined in section 8 and both fractionated together.

The normal hydrochloric acid solution is again partly neutralized by addition of sodium hydroxid (to fourth-normal or less) and is shaken out with a mixture of 3 volumes carbon tetrachlorid and 1 volume dichlorhydrin to extract the lower sulphonated triphenylmethane dyes. These may be obtained in water solution again, by washing out with water after adding more carbon tetrachlorid.

SEC. 12. The acetic acid solution obtained by the procedure described in section 10 will contain the chief part of any monosulphonated monazo dyes present. Such colors may be further fractionated, with amyl alcohol and normal sodium carbonate, with ether and dilute hydrochloric acid, etc.

The coloring matters of this group may appear in small proportion in the fractionation described in section 8, and obviously the similar solutions there described may be combined with the acetic acid solution obtained as described in section 10.

SEC. 13. The main part of the eosin dyes, and of unsulphonated water-soluble acid colors in general, will be found in the sixty-fourth-normal sodium hydroxid solution obtained by the extraction described in section 10. A large proportion of the natural coloring substances also appear here.

The eosin dyes may be fractionated between normal sodium hydroxid and amyl alcohol or amyl alcohol gasoline mixture (3:1).

The acid dyes also having basic tendencies, as Fluorescein (No. 510), Metanil Yellow (No. 95), etc., differ from the others in being extracted from strongly acid solutions in smaller amounts than from weakly acid solutions, and this property offers a suitable means for their separation (pages 27-28). These colors, as already pointed out, may be obtained, though in most cases in very small proportion, in the amyl alcohol gasoline solution obtained by the extraction described in section 8.

SEC. 14.—The residue mentioned in section 10 is moistened with a small drop of alcohol, and then some ether and sixty-fourth-normal hydrochloric acid are added. The mixture is poured into a separatory funnel and is shaken. The aqueous layer is drawn off, and if dyes coloring the aqueous solution were present, the ether is washed a few times further with the sixty-fourth-normal acid, to remove them. The acid solution contains the rhodamins, perhaps also some of the basic azo colors.

The ether solution if colored is now washed a few times with four-normal hydrochloric acid, the washings being neutralized at once and reserved for treatment according to section 15.

The ether is finally washed a few times with water to remove acid; then it is taken to dryness on the steam bath and the residue treated according to section 16.

SEC. 15.—This group, consisting of oil-soluble colors, may be further separated by taking up the dye in gasoline or petroleum ether from the neutralized solution obtained as described in section 14, and fractionating from this solvent with methyl alcohol, 70 per cent or above. (See pages 32-33.) Ortho-tolueneazo- β -naphthylamin suffers decomposition rather rapidly in strongly acid solutions. On the other hand, both it and the lower benzene homologue, when their ether solutions are shaken with acid, are extracted, but slowly, the amount of color removed from the ether depending on the time of contact of the two layers. The substance in the ether layer would thus seem to undergo rearrangement before forming the water-soluble salt, but one or both forms suffer complete decomposition by prolonged standing with the acid.

SEC. 16.—The residue containing Sudans, etc., may be treated with measured quantities of methyl alcohol, water, and sodium hydroxid in the proportions necessary to bring the mixture to fourth-normal alkalinity in 80 per cent alcohol; the solution then may be shaken out with gasoline. Quinolin Yellow and α -naphthol derivatives remain chiefly in the alkaline solvent. The petroleum ether is washed again, if necessary, with the same mixture, then treated as described in section 17.

SEC. 17.—The Sudans and similar dyes of this group may be further separated with gasoline and 90 per cent methyl alcohol. They may be separated from troublesome accompanying oily impurities, by shaking out the gasoline solution with 85 per cent phosphoric acid to which has been added from 10 to 20 per cent of concentrated sulphuric acid, though some dye is likely to be destroyed in this treatment.

Like the colors described in sections 15 and 16, these dyes are almost quantitatively removed from gasoline solution by 90 per cent phenol. The phenol may, of course, be dissolved in alkali and the color again taken up with ether or gasoline, effecting a separation from some impurities.

SEC. 18.—When Naphthol green B is present the salt solution should not be made strongly acid since small amounts of this dye decompose quickly in acid solution. When its presence is suspected, the neutral salt mixture may be first extracted with dichlorhydrin washed once with benzene to remove the dissolved solvent, made half normal with hydrochloric acid, and then shaken out with anilin. (It is best to add the solvent before the acid.) From the anilin solution the dyes may be fractionated by shaking out with 5 or 6 per cent salt solution which contains hydrochloric acid varying from fourth-normal to sixty-fourth-normal.

No outline in the form of a key can be so useful as a table of solubilities. The solvents and the order in which they are to be used obviously may be varied when the analyst has information regarding the source and appearance of a sample. For example, in the case of the red color solution obtained from commercial cocktail cherries known to be ordinarily colored with Erythrosin, it would be better to make such solution acid and shake it out with ether first, the complete extraction of the dye indicating at once the absence of all excepting one group of colors.

In the examination of dye solutions the analyst of some experience often will prefer (especially with colors extracted from acid solutions by amyl alcohol) to wash the organic solvent extract with successive small portions of water instead of with hydrochloric acid or other aqueous solvent of definite concentration. In the case of an acid amyl alcohol extract much hydrochloric acid is taken up by the solvent along with the coloring matter. The acid is washed out rather slowly, and as a result of this gradually decreasing acidity of the washing coloring matters of different solubility will appear in maximum amount in different fractions, the order being apparent from the solubility tables. With binary mixtures usually some of each color is thus obtained pure enough for identification.

The writer prefers, after fractionating the colors into the main groups as just described, to try the bromin test (page 47). The behavior with acids has already been seen in the course of the separation, and that with alkali can be quickly ascertained. Ordinarily these will indicate the fraction to contain but one coloring matter. This is then dyed out from a portion of the solution, and its shade and reactions on the fiber with reagents are compared with standards or with statements in the tables, in which, to facilitate comparison, the dyes have been arranged in the order of their solubility. Since the color

changes produced in dilute dye solutions, by addition of acids and alkalies, are closely parallel to those shown by the same reagents on the dyed wool, a single table indicating the reactions on the fiber is sufficient in practice.

Even when the tests have indicated that the fraction still contains a mixture of dyes, they will have shown, in most cases, the absence of many colors of the group, and will have indicated positively which colors are probably present.

ABRIDGED PROCEDURE FOR PERMITTED DYES ONLY.

A convenient abridgment of the fractionation procedure, suitable when it seems probable that only permitted dyes are present, is the following:¹

The solution or well divided solid matter containing the color is treated with one-half its volume of concentrated hydrochloric acid and is then extracted a few times with amyl alcohol. (For precaution concerning concentration in examining commercial food colors, see page 4.) The alcohol extracts are combined, then washed with four or five portions of fourth-normal hydrochloric acid, or until this solvent extracts very little color. These washings will contain any Indigo Carmine, Tartrazin, and Amaranth which were present in the alcohol solution. Indigo Carmine is removed from the amyl alcohol somewhat more readily than are the other two dyes. With ordinary concentrations little or no Ponceau will be removed.

The amyl alcohol is then measured, if necessary, treated with an equal volume of petroleum ether or low-boiling-point gasoline, and again washed several times with fourth-normal hydrochloric acid to extract Ponceau 3 R and Naphthol yellow S. Or, without dilution with gasoline, it may be washed with 5 per cent salt solution until these two dyes are taken out. After the Ponceau and Yellow have been removed the amyl alcohol, which contains an equal volume of gasoline, is washed a few times with water, thus extracting Orange I. After the removal of this dye the solution, although perhaps appearing almost colorless, is shaken out with a very dilute caustic soda solution to remove Erythrosin.

If considerable Orange I is present, some of it may contaminate the washings containing the Ponceau 3 R and Naphthol yellow S, especially when these have been separated by means of fourth-normal hydrochloric acid after addition of gasoline.

The fourth-normal hydrochloric acid washings of the amyl alcohol may contain Indigo Carmine, Amaranth, and Tartrazin, their appearance in most cases indicating which of these dyes may be present. Instead of attempting to separate the dyes by fractionation the fourth-normal hydrochloric acid solution may be evaporated to dryness,

¹ See also Price, U. S. Dept. Agr., Bur. Anim. Ind. Circ. 180.

the residue dissolved in water, and the constituent colors identified in portions of this solution. A portion of the slightly acidified solution may be warmed, and a few decigrams of urea and then one or two drops of sodium nitrite solution added. Indigo Carmine is converted into the pale yellow Isatinsulphonate, while the other dyes are but little affected. The Isatin compound is not ordinarily present in sufficient concentration to tint the solution, but it differs from Tartrazin also in being much less readily extracted by amyl alcohol from strong acid solution (less than one-half from four-normal acid). Amaranth is much more quickly attacked by most reducing agents than Tartrazin, and may be destroyed in mixtures containing Tartrazin by treating the neutral or faintly acid solution at room temperature with sodium hydrosulphite. (In the presence of sodium carbonate the reduction of Tartrazin takes place still more slowly.) The reagent should be added very carefully, either in dilute solution or as the powder, sufficient time being allowed after each addition for the reduction to take place. When the color shows that the Amaranth has been completely destroyed, the mixture should be shaken at once with air to oxidize the slight excess of hydrosulphite before it can react further on the Tartrazin. To separate the Indigo Carmine another portion of the neutral or faintly acid solution may be heated to boiling and hydrosulphite added very carefully, a few particles at a time, until all the dyes are reduced. On shaking with air the Indigo Carmine is quickly re-formed.

The fourth-normal acid solution (or the salt solution) containing the Ponceau and Naphthol yellow S is treated with enough hydrochloric acid to make it about twice normal, and is shaken out a few times with washed ethyl acetate.¹ The yellow is removed from the combined ethyl acetate extracts by shaking out with water. It must always be remembered that Naphthol yellow S is almost colorless in strongly acid solutions, and its absence in washings, etc., must never be assumed until they have been made alkaline. The Ponceau 3 R is finally separated from the acid solution by shaking the solution with amyl alcohol, then washing out the dye from this extract with a few small portions of water.

When (with mixtures containing Orange I) the washings of the ethyl acetate, which should contain only Naphthol yellow S, become redder with alkalis, they should be combined, made fourth-normal with hydrochloric acid, and the contaminating orange removed by extracting with amyl alcohol gasoline mixture (1:1). Or they may be treated with one-fifth their volume of concentrated hydrochloric acid and the dyes extracted by shaking once with amyl alcohol. From this solution the yellow may be removed by washing with several portions of 5 per cent salt solution.

¹ Used instead of amyl acetate as suggested by Loomis, U. S. Dept. Agr., Bur. Chem. Bul. No. 162, p. 57.

The original mixture, from which the seven colors described were separated by adding acid and shaking out with amyl alcohol, may still contain Light green S F yellowish, which will be colorless or nearly so in the acid solution. To separate this dye the mixture is treated with strong ammonia or potassium hydroxid solution until slightly alkaline, then neutralized with acetic acid. Any green that is present will now be apparent by the color of the mixture and may be extracted by shaking with a few small portions of dichlorhydrin. After washing the dichlorhydrin extract with a little water it should be diluted with several volumes of benzene or carbon tetrachlorid. The dye is then taken out with water.

Besides the well-known reactions of acids and alkalies on the dyed fiber or with the solution, a few tests may be mentioned as best suited for the quick characterization of the different colors obtained in this separation. Indigo Carmine is extracted in rather small proportions from acid aqueous solutions by dichlorhydrin, differing in this respect from nearly all the other common blue dyes. The bromin test (p. 47) and the reactions with acids and alkalies usually are sufficient for the identification of Tartrazin after its separation. New Coccin (No. 106) and Ponceau 6 R (No. 108) are the only other common red dyes of solubility similar to that of Amaranth. Both are yellowish in shade, the former markedly so. Ponceau 3 R, when treated with barium chlorid and enough sodium acetate to neutralize any free hydrochloric acid present, gives a bluish-red flocculent precipitate, the supernatant liquid being left practically colorless. Ponceau 2 R gives a carmine red precipitate; but most other red dyes of similar solubility do not form particularly insoluble barium salts. Naphthol yellow S in solutions treated with an excess of ammonia or sodium carbonate becomes intensely rose colored on addition of sodium hydro-sulphite, the color gradually fading again as complete reduction takes place. Orange I is well characterized by its solubility and behavior with acids and alkalies. Erythrosin is perhaps best further identified by a test for iodine. Some of the color solution containing a slight excess of alkali is evaporated to dryness, the residue heated to redness, and the ash taken up with water and acidified with sulphuric acid. Iodine may be tested for in the usual ways, such as with carbon tetrachlorid and a small drop of sodium nitrite, or with starch paste and an oxidizing agent. It is useless to test for iodine with very small amounts of dye, but in most cases sufficient coloring matter will be available to give satisfactory results.

TREATMENT OF SOLUTION RESERVED FOR TESTING FOR NATURAL COLORING SUBSTANCES.

If coal-tar dyes have been found, the treatment for their separation will have given much information as to natural colors that may be present. Many of the natural colors will have been separated in

fairly pure condition by the fractionation and the solutions obtained will be ready for identification tests. Obviously, no essential difference exists between these and the so-called coal-tar colors; as a class, however, they show much less tendency to dye wool than do the common synthetic colors, and in addition are in many cases so sensitive to alkalis as to be completely destroyed in the double-dyeing test; i. e., by dyeing, stripping the fiber with dilute ammonia, acidifying, and dyeing again. The preliminary dyeing with wool described on page 8 serves fairly well in practice as a first indication of the course to be followed; but when for some reason the results obtained are not decisive, the general treatment for coal-tar colors with immiscible solvents should be carried out with consideration of solubilities of the coloring matters described in the footnotes in the tables of solubilities. In the dyeing test not only are certain synthetic dyes, especially Auramin, unstable under the conditions of treatment and likely to be destroyed; but, on the other hand, Archil, and to a lesser extent other natural colors, may give well-marked dyeings.

The crude products constituting the commercial natural coloring matters in most cases are mixtures containing several closely related chemical individuals. These may have different solubilities, but usually they contain the same chromophore groups, and are of closely similar shade. In practice, the analyst will scarcely attempt a full separation, but having identified the coloring matter in one fraction, can judge as to the likelihood of the other substances present being derived from the same source.

The natural colors as a class do not contain strongly acidic groups, and their distribution ratios between immiscible solvents do not show wide variations with the acidity of the latter, at least not within convenient limits. The coloring matters of cochineal and turmeric give less trouble than the others, partly because they are less heterogeneous.

When coal-tar dyes are absent, and it is desired to fractionate with immiscible solvents, it is best to begin extraction with neutral solutions; perhaps first using ether (petroleum ether is better when chlorophyll and the accompanying leaf colors are to be separated). The final extraction may be made with amyl alcohol from acid solution, but it is of no advantage to have the acidity high, not, perhaps, above thirty-second normal. The anthocyanins which constitute the coloring matters of the common red fruits and flowers are glucosids, and are extracted from acid solution only in very small amount by amyl alcohol and similar solvents. Their neutral solutions may be treated with excess of lead acetate solution (normal salt) when practically all of the glucosid will be precipitated. This mixture may be centrifuged and the precipitate washed in the centrifuge tubes with several portions of water until sugar and similar soluble substances

TABLE 1.—*Extraction of coloring substances from aqueous solutions by immiscible solvents.*

Statements refer in all cases to the amount of dye taken up by the organic solvent when this is shaken with an equal volume of the 0.01 per cent solution of the coloring matter in the various aqueous liquids.

The coloring matters are designated by numbers as given in the tables in "A Systematic Survey of the Organic Coloring Matters." By A. G. Green. Founded on the German of Drs. G. Schultz and P. Julius. Second edition, London and New York, 1904.

For names and references to other large published tables, see page 56.

Numbers denoting permitted dyes are in bold-faced type; natural colors included in the body of the tables are in italics. The numbers of the nonpermitted dyes more commonly found in foods are starred, three stars indicating those dyes most frequently used.

Serial numbers are given in the first column to facilitate reference to the tables of properties, pages 37, 46, 50, and 51.

No.	Coloring matter.	Color of water solution.	Amyl alcohol and approximately normal (3-5 per cent) sodium chlorid solution.	Amyl alcohol and hydrochloric acid solutions of varying normality.	Amyl alcohol gasoline mixture (1:1) and dilute hydrochloric acid.	Ether.	Dichlorhydrin and N sodium chlorid solution.	Other data.
1	*462	Crimson; with HCl, little change.	Little or none extracted.	Little or none extracted from acid of concentration 4 N or less.	Little or none extracted from acid of normal concentration or more dilute.	Little or none extracted from acid, neutral, or alkaline solutions.	Little extracted.	
2	434	(Green; strongly acid solution, brownish yellow to colorless according to dye concentration.)					Larger part or almost all extracted from 5-20 per cent salt solution.	With a mixture of 1 volume washed dichlorhydrin, 3 volumes carbon tetrachlorid, and with N/64 acetic acid: 435, 439, very little extracted; 491, little extracted; 440, one-half or more extracted.
3	435							
4	*436	Greenish blue; with HCl, same reaction as 435.						
5	439	Blue; with HCl, same reaction as 435.						
6	491	Greenish blue; with HCl, same reaction as 435.						
7	440	Blue; with HCl, same reaction as 435.	Less than one-half extracted.					
8	**402	Blackish blue; with HCl, little change.	Little extracted.					
9	**108	Magenta red; with HCl, little change.	Little or none extracted.	4 N, larger part extracted; N, little extracted.			Little or none extracted.	
10	***8	Yellow; with HCl, red.		4 N, larger part extracted; N, one-half or less extracted; N/4 and below, little or none extracted.				With 8 N H ₂ SO ₄ and amyl alcohol gasoline mixture (equal volumes): 8, little extracted; 682, larger part not extracted; 89, more than one-half extracted; 107, 106, and 94, larger part extracted.
11	**89	Yellow; with HCl, red.						With anilin and acid, 5 per cent
12	692	Blue; with HCl, little change.						
13	399	Yellow; with HCl, little change.						
14				4 N, almost all extracted; N, larger part extracted;				

15	***106	Scarlet red; with HCl, little change.	N/4 and below, little or none extracted.	sodium chlorid solution (normality stated is that of the HCl before shaking); N/2, 692, 107, and 94 almost all extracted; N/4, 94, less than one-half extracted; 8, 89, 692, 107, and 338, almost all extracted; N/16, 94, little extracted; 89, less than one-half extracted; 692, about one-half extracted; 106, 107, more than one-half extracted; 338, about one-half extracted; N/64, 107, 106, 94, and 338, little or none extracted.
16	107	Magenta red; with HCl, little change.		

Most of the dyes described on these two pages are usually accompanied by considerable amounts of subsidiary products, especially of substances similar chemically but of a different degree of sulfonation. Caramel and the glucosids constituting the coloring matters of most red fruits resemble Acid Magenta (No. 462) in being extracted in small proportion only, by amyl alcohol or dichlorhydrin from the various aqueous solutions.

TABLE 1.—*Extraction of coloring substances from aqueous solutions by immiscible solvents*—Continued.

No.	Coloring matter.	Color of water solution.	Amyl alcohol and approximately normal sodium chlorid solution.	Amyl alcohol and hydrochloric acid of varying concentration.	Amyl alcohol gasoline mixture (1:1) and hydrochloric acid of varying normality.	Ether.	Dichlorhydrin-carbon tetrachlorid mixtures (equal volumes; also 1 volume dichlor. +3 volumes CCl ₄).	Washed ethyl acetate and 2N hydrochloric acid.
17	94	Yellow; with HCl, no change.	Little or none extracted.	As stated for 106 and 107, p. 23.		Little or none extracted by ether from neutral acid or alkaline solutions.	Little or none extracted by mixture 1:1 or by mixture 1:3, from N/4 HCl or N/64 acetic acid.	Very little or none extracted by ethyl acetate.
18	*398	Green; with HCl, gradually yellow.		Similar to dyes just preceding, but rapidly decomposed.				
19	605	Red; with HCl, little change.		4 N and N; larger part extracted; N/4 less than one-half extracted.				
20	604	Red; with HCl, little change.			4 N, larger part extracted; N and below, little or none extracted.			
21	188	Violet; with HCl, bluer.		N and above, almost all extracted; N/4, about one-half extracted; N/16 and below, little extracted.				
22	**14	Orange; with HCl, no change.		4 N and N, chief part extracted; N/4, more than one-half; N/16, less than one-half.	4 N, larger part not extracted; N, little extracted.			
23	21	Magenta red; with HCl, slightly yellower.			Behaves as stated for 14 and 188.			
24	318	Blue; with HCl, little change.		N and above, almost all extracted; N/4, larger part extracted; N/64, less than one-half extracted.	4 N, almost all extracted; N, larger part not extracted; N/16 and below, little or none extracted.			
25	20	Magenta red; with HCl, no change.						
26	93	Bluish red; with HCl yellower.						
27	***480	Blue; with HCl, slightly paler.			4 N, less than one-half extracted; N and below, little extracted.			

28	*53	Scarlet; with HCl, little change.	N/4 and above, almost all extracted; N/64, one-half or more extracted.	N, one-half or less extracted; N/16 and below, little or none extracted.		
29	*55	Scarlet; with HCl, little change.	The same as above, but large part extracted at N/64.		As stated for 56, 62, etc., p. 27.	Larger part extracted.
30	105	Scarlet; with HCl, little change.				
31	4	Yellow; with HCl, almost colorless.				
32	**706	Orange red; with HCl, little change.				
						Little extracted.

TABLE 1.—*Extraction of coloring substances from aqueous solutions by immiscible solvents*—Continued.

No.	Color- ing matter.	Color of water solution.	Amyl alcohol and approximately normal (5-6 per cent) sodium chlo- ride solution.	Amyl alcohol and hydrochloric acid of varying concen- tration.	Amyl alcohol gas- line mixture (1:1) and hydrochloric acid of varying concentration.	Ether.	Dichlorhydrin- carbon tetra- chlorid mixtures.	Washed ethyl acetate and 2 N hydrochloric acid.	Amyl alcohol gas- line mixture (1:1) and dilute sodium hydroxid solution.
33	56	Cherry red; with HCl, no change.	Little or none ex- tracted.	N/16 and above, almost all ex- tracted; N/64, larger part ex- tracted.	N, more than one- half extracted; N/16 and below, little or none ex- tracted.	Little or none extracted by from acid, neutral, or alkaline so- lutions.	Little or none extracted by mixture 3 vol- umes CCl ₄ and 1 volume washed dichlorhydrin, from N/64 acetic acid.	Little ex- tracted.	Little or none extracted from N/64 NaOH solu- tions.
34	*62	Bluish red; with HCl, little change.							
35	*64	Scarlet, with HCl, slightly darker.							
36	*65	Magentared, with HCl, little change.							
37	**103	Same reaction as 65.							
38	139	Brown; with HCl, little change.	Intermediate in behavior be- tween preced- ing and succeed- ing groups. Of most of the dyes, more than one- half is extracted.	Almost all ex- tracted at N/64 and above.	N, larger part ex- tracted; N/16, larger part not extracted; N/64, little extracted.				
39	164	Orangered, with HCl, darker; finally violet.							
40	**667	Yellow; with HCl, no change.							
41	*169	Scarlet; with HCl, darker; finally violet.							
42	163	Orangered; with HCl, darker.							
43	170	Red; with HCl, darker; finally blue-violet.							
44	84	Orange yellow; with HCl, little change.							
45	146	Cherry red; with HCl, darker; finally blue-violet.							
46	287	Violet; with HCl, little change							
47	78	Red.							
48	**710	Violet.							

The coloring matters described on the lower half of this page are soluble with difficulty in both layers with most acid mixtures. This is especially true of Nos. 78, 710, and 287, which may be precipitated to some extent.

[illegible]

The dyes described on this page are rather difficultly soluble with acid mixtures.

No.	Color- ing matter.	Color of water solution.	Amyl alcohol and approxi- mately normal (5-6 per cent) sodium chlorid solution.	Amyl alcohol and dilute hydrochloric acid.	Amyl alcohol gasoline mixture (1:1) and dilute hydrochloric acid.	Amyl alcohol gasoline mixture (1:1) N/64 acetic acid (mixture, 1:1).	Ether and dilute hydro- chloric acid.	Amyl alcohol gasoline mixture (1:1) and N/64 sodium hydroxid solution.	Ether and dilute sodium hydroxid solution.	Amyl alcohol and normal sodium hydroxid solution.	Amyl alcohol gasoline (3:1) and normal sodium hydroxid solution.
81	2	Orange yellow.	Almost all ex- tracted.	Almost all ex- tracted at normalities 1/64 and above.	Almost all ex- tracted.	Almost all ex- tracted.	Almost all ex- tracted at normalities 1/64 and above.	Little extracted (6 larger part not extracted).	Little extracted, N to N/64 ex- cept 6; N, al- most all ex- tracted; N/64, more than one- half extracted; 3, less than one-half ex- tracted from N.		
82	*3	Yellow.									
83	6	Orange yellow.									
84	534	Alkaline solution, Violet.									
85	**707	Alkaline solution, reddish brown.									
86	*10	Alkaline solution, orange.									

Coloring matters of fustic, of quercitron, of Persian berries after hydrolysis, and of alkanet, have similar properties. They are extracted in large proportion by amyl alcohol and by amyl alcohol gasoline mixture (equal volumes) from N/64 acetic acid or from hydrochloric acid, N/64 to N; also by ether from N/64 hydrochloric acid. They are not extracted in large proportion by amyl alcohol gasoline mixture or by ether from N/64 sodium hydroxid solutions. Annatto, not alkali treated, behaves similarly but is extracted in large proportion from N/64 sodium hydroxid by amyl alcohol gasoline mixture (1:1).

Coloring matters of barwood, camwood, and sandalwood resemble Nos. 483 and 510 somewhat in behavior, but are less soluble in aqueous solvents. They are extracted almost completely by amyl alcohol from salt solution, and by amyl alcohol, amyl alcohol gasoline mixture (1:1), and ether from N/64 hydrochloric acid. They are not extracted by amyl alcohol gasoline mixture or ether from N/64 sodium hydroxid. From 4 N hydrochloric acid the chief part of the coloring matter is not extracted by ether; from N hydrochloric acid the chief part is extracted. The coloring matter of brazilwood is similar but relatively somewhat more soluble in aqueous solvents. That of logwood is also similar, but still more soluble in the water solutions. It is almost all extracted by amyl alcohol from salt solution or N/64 hydrochloric acid. The larger part is extracted by amyl alcohol gasoline mixture (equal volumes) from N/64 hydrochloric acid. The chief part is not extracted by ether from N/64 hydrochloric acid and very little from 4 N hydrochloric acid. It is extracted in very small proportion from alkaline solution (N/64 sodium hydroxid) by ether or amyl alcohol gasoline mixture.

TABLE 1. — *Extraction of coloring substances from aqueous solutions by immiscible solvents.* — Continued.

No.	Color- ing matter.	Color of water solution.	Amyl alcohol and sodium chloride normal (5:6 per cent.) sodium chloride solution.	Amyl alcohol and dilute hydro- chloric acid.	Amyl alcohol gasoline mix- ture (1:1) and dilute hydro- chloric acid (N/64).	Amyl alcohol gasoline (1:1) and N/64 acetic acid.	Amyl alcohol gasoline (1:1) and N/64 sodium hydroxide.	Ether and dilute acid (N/64 hydro- chloric acid or acetic acid).	Ether and N/64 sodium hydroxide.	Dichlorhydrin carbon tetrachloride in 1X- acetic acid.
87	*468	Violet.	Nearly all extract- ed.	4 N, very little ex- tracted.	Little extract- ed.	Little extract- ed.	Little extract- ed.	Little or none extracted.	Little or none extracted.	Almost all extract- ed by mixture of equal volumes of solvents. By mixture 3 vol- umes CCl ₄ 1 vol- ume C ₆ H ₅ OC ₂ H ₅ , one-half or more extracted.
88	464	Violet.		N/64, almost all ex- tracted.	More than one-half extracted.	More than one-half extracted.	Chief part ex- tracted.			
89	438	Green.		N/64, almost all ex- tracted.	Larger part not extracted.	Larger part not extracted.	Larger part not extracted.			
90	**433	Green.		Partially extract- ed.						
91	442	Blue.		Almost all extract- ed at N/64.	Some extract- ed; remainder precipitated.					
92	476	Blue.						Little or none extracted; precipitated by HCl.		
93	240	Red.	Extracted from neutral or slight- ly alkaline solu- tion.	Dye precipitated.	Dye precipi- tated.	Precipitated.	Not ex- tracted.			
94	277	Brownish red.	Most of dye pre- cipitated.	Most of dye pre- cipitated; rest extracted.						
95	562	Alkaline solution blue to green.								
96	658	Yellow.	Almost all extract- ed.		Very little extracted.	Very little extracted.		Little or none extracted.		
97	496	Red.								
98	650	Blue.								
99	639	Violet.								
100	*584	Red.								
101	**448	Crimson.								

No.	Color- ing mater.	Color of water solution.	Amyl alcohol and normal so- dium chlorid solution.	Amyl alcohol and hydrochloric acid of varying concentration.	Amyl alcohol gasoline mixture (1:1) and N/64 hydrochloric acid.	Amyl alcohol gasoline mixture (1:1) and N/64 acetic acid.	Ether and dilute hydro- chloric acid N/64 and above.	Ether and dilute acetic acid.	Ether and dilute sodium hydroxid solution.
102	**425 426	Yellow. Yellow.	Almost all ex- tracted.		Chief part not ex- tracted.	Little extracted.	Little or none extracted.		Almost all extracted at N/64, but easily decomposed.
103					Less than one- half extracted.	Chief part not ex- tracted.			N, chief part extracted.
104	**451 452	Violet. Violet.			Chief part not ex- tracted.	Less than one-half extracted.		N, little extracted; N/64, as below.	Almost all extracted, N to N/64.
105					Very little ex- tracted.	More than one-half extracted.		N, larger part, not extracted; N/64, chief part extracted.	
106	**427 428	Green. Green.			Chief part not ex- tracted.				Almost all extracted at N.
107					More than one- half extracted.	Chief part ex- tracted.			Almost all extracted, N to N/64.
108	*197 *201	Brown. Brown.							
109									
110	17 18	Orange. Orange.							
111									
112	505 499	Bluish red. Bluish red.							
113									
114	**504 502	Bluish red. Bluish red.				Almost all extracted.			
115									

128	*143	Benzeneazobenzeneazo- β -naphthol.	Red.							
129	*	Ortho-tolueneazo-o-toluene-azo- β -naphthol.	Red.						Chief part extracted.	Little extracted.
130	31	Para-nitrobenzeneazo-o- β -naphthol.	Orange.						Chief part not extracted.	

Ortho-tolueneazo- β -naphthylamin is rather rapidly destroyed in strongly acid solutions.

have been removed. The precipitate may then be dissolved in hydrochloric acid of 10 or 15 per cent strength. After whirling again in the centrifuge to separate the lead chlorid thrown out of the solution, the clear red liquid is shaken out once or twice with amyl alcohol to remove various extractives soluble in this substance. It may then be boiled for a short time, by which means the glucosid is hydrolyzed, the derived coloring matter, or anthocyanidin, being produced. This may now be extracted and obtained in fairly pure solution by shaking out with amyl alcohol. The anthocyanidins, according to Willstaetter,¹ are oxonium bases, containing also acidic phenolic groups. They are not very readily soluble in amyl alcohol though relatively more so than in aqueous liquids.

The coloring principles of saffron and of Persian berries also consist chiefly of glucosids, though the lead salts of these are relatively more soluble. These glucosids also are readily hydrolyzed by boiling with acid, but the change in case of saffron is attended with destruction of much coloring matter, at least when the hydrolysis is carried out in the ordinary manner, with free access of air. Berberine is said to be the only common natural basic coloring matter and it is seldom, if ever, found in food products.

By extraction from neutral solutions with ether, the leaf pigments (identical or similar colors are also found in egg yolk,² fats and oils,³ carrots, and tomatoes⁴) are taken up. They are removed from this solvent by washing with dilute alkali.

Coloring matters of alkanet, annatto, turmeric, and of the red dye-woods (sandalwood, camwood, and barwood) are very readily and completely extracted by ether from slightly acid solutions. The flavone coloring matters of fustic, of Persian berries (after hydrolysis), and of quercitron, also the coloring matter of brazilwood and the green derivatives formed from chlorophyll by alkali treatment, are taken up in very large proportion by ether from slightly acid solutions.

The coloring matters of logwood, of archil, of saffron, and of cochineal are extracted in relatively small amount by ether from slightly acid solutions, but are largely taken up by amyl alcohol.

Caramel and the anthocyanins constituting the red coloring matters of most common fruits are extracted in relatively small proportion by amyl alcohol from acid solutions. Ammoniacal cochineal (carmine) is similar, but the ordinary coloring matter is readily re-formed by standing with hydrochloric acid.

¹ Sitzb. kgl. Preuss. Acad. 12 (1914), 402-411. For further papers by Willstaetter and coworkers see Liebig's Ann. 408 (1915), 1-158.

² Willstaetter and Escher, Zeit. Physiol. Chem. 76, (1912), 214.

³ See Palmer and Eckles, Mo. Sta. Research Bul., Nos. 9, 10, 11, 12.

⁴ Willstaetter and Stoll, Untersuchungen ueber Chlorophyll, Berlin (1913).

IDENTIFICATION OF COLORING SUBSTANCES.

COAL-TAR DYES.

The coloring matters are usually obtained by the fractionation dissolved in various aqueous or organic solvents, but free from non-volatile substances. Neutral solutions suitable for certain tests are most easily obtained by evaporating to dryness and taking up the residue with water or other suitable solvent.

It is not intended to discuss here the innumerable tests that may be used for the individual dyes. There are described a limited number only of general procedures which are quickly and easily carried out, and the chemistry of which is for the most part understood. These tests are sufficient for identification in ordinary cases.¹

For ready comparison of colors of similar solubility, it is convenient to have tables of properties in which the arrangement is based on the solubility. The familiar reactions of reduction and of color changes with reagents applied to the dyed fiber are given in this order in Tables 2 and 3, these tests being made as follows:

*Reactions on dyed fiber.*²—Small pieces or shreds of the dyed wool are distributed on the porcelain plate and are thoroughly moistened with the reagents. The fiber must be dry or nearly so, and must have been dyed in a fairly pure solution of the color, since colorless organic impurities may easily obscure the reaction.

*Reduction and subsequent reoxidation.*³—The neutral color solution is treated with a few particles of powdered sodium hydrosulphite, conveniently dropped in from a small spatula. If no color change is seen at once, the mixture is warmed somewhat and more reagent added, carefully avoiding excess, however. If reduction, shown by disappearance of the color, takes place, the solution is thoroughly shaken with air, and should this not bring back the dye, it is warmed and allowed to stand a few minutes. Finally, if remaining practically colorless, a little powdered potassium persulphate is dropped in. A slight yellowish or brownish tint produced by air or especially by the potassium persulphate is disregarded.

In regard to the tests on the dyed wool, it may be said that the dyeings obtained from colors in food products are necessarily variable in depth and usually paler than those used as a basis for standards. With the very small amounts of color available, it is impossible to

¹ For the identification of the simpler azo dyes by reduction, separation of the reduction products and characterization of these by coupling with diazo compounds, by condensation with nitrosodimethylaniline, and by diazotization, see especially Witt, *Ber. Chem. Ges.* **19** (1886), 1719, and **21** (1888), 3468. Properties of the various amines, aminophenols, and their sulphonic acids are summarized by Heumann (Friedländer, Schultz), *Die Anilinfarben*. Braunschweig, 1888-1906.

² The tables are made to correspond as nearly as possible with those of U. S. Dept. Agr., *Bur. Chem. Cir. No. 63*, 0.5 per cent dyeings and reagents of similar concentration being used.

³ Hydrosulphite and persulphate are the reagents advocated by Green, Yoeman, and Jones, *J. Soc. Dyers & Colorists* **9** (1905), 236; also Green, *Identification of Dye Stuffs*, Leeds (1913).

make dyeings to any convenient standard depth, and descriptions can only indicate in a general way what may be expected. Not only will the appearance of the dyed wool under the influence of different reagents vary somewhat with the concentration of the dye present, but the shade of the dry fiber also may vary with the concentration of the dye. For example, dyeings from some of the oranges are almost yellow when only a little color is present, but are a much redder shade when more dye is used.

Color changes similar to those taking place on dyed fibers are produced in most cases by the given reagents in solutions of the dyes, and the conditions are under much more exact control. So in some cases it is advantageous to compare the solution of the dye under examination with solutions of known colors, all being brought as nearly as possible to the same dye concentration, and to the same acid or alkali normality. An exact statement of shade can be given best by spectrophotometric data (according to Vierort's method) under prescribed conditions of temperature and concentration. This is desirable in some cases, as when dealing with mixtures of Ponceau 2 R and Ponceau 3 R. For the somewhat related and more rapid spectroscopic method of measuring the spectral position of maximum light absorption in dilute solutions, the treatises of Formanek, of Formanek and Grandmougin, and of Mulliken may be consulted. However, the "spot reactions," if not of the greatest exactness, are sufficiently exact for most purposes, and are especially convenient in inspection work, where it is well to keep a specimen of the color dyed on cloth.

TABLE 2.—*Behavior of dry colors or of dyed fibers with reagents.*

[Numbers denoting permitted dyes are in boldfaced type; natural colors are in italics. Dyes common in foods are starred, three stars indicating those dyes most often found.]

Reactions of dyed wool with reagents.								
Color- ing mater.	Color of sulphuric acid solution.	Color of dyed wool.	Concentrated hydro- chloric acid.	Concentrated sulphuric acid.	10 per cent sodium hydroxide solution.	Ammonia solution, specific gravity, 0.95.		
1	***462	Yellow.	Nearly decolorized.	Yellow	Decolorized.	Decolorized.		
2	434	Yellow.	Yellow.	Orange or pale brown.	Decolorized.	Decolorized.		
3	435	Yellow.	Yellow.	Orange or pale brown.	Decolorized.	Decolorized.		
4	*436	Yellow.	Yellow.	Orange or pale brown.	Little change (slightly darker).	Little change (slightly darker).		
5	439	Yellow.	Yellow.	Orange or pale brown.	Yellow olive.	Redder.		
6	491	Yellow.	Yellow.	Orange or pale brown.	Little change.	Little change.		
7	440	Yellow.	Yellow.	Orange or pale brown.	Little change.	Little change.		
8	**602	Deep blue.	Dull bluish	Dull greenish.	Pale brownish red.	Pale reddish.		
9	**108	Violet.	Bluish red.	Violet.	Brown.	Orange red.		
10	***8	Brownish yellow.	Red.	Orange.	Little change.	No change.		
11	9	Brownish yellow.	Red.	Orange.	Little change.	No change.		
12	**89	Violet red.	Bluish red.	Bluish red.	Little change.	Little change.		
13	692	Violet blue.	Slightly darker.	Slightly darker.	Greenish yellow.	Greenish blue.		
14	399	Reddish brown.	Yellowish, dull.	Reddish brown.	Orange.	Orange.		
15	***106	Red violet.	Red.	Violet red.	Yellowish brown.	Orange red.		
16	107	Violet.	Slightly darker.	Violet to brownish.	Dull brownish.	Little change.		

TABLE 2.—*Behavior of dry colors or of dyed fibers with reagents—Continued.*

Reactions of dyed wool with reagents.								
No.	Color- ing mater.	Color of sulphuric acid solution.	Color of dyed wool.	Concentrated hydro- chloric acid.				
				Concentrated sulphuric acid.	10 per cent sodium hydroxid solution.	Ammonia solution, specific gravity, 0.95.		
17	94	Yellow.	Yellow.	Slightly darker.	Slightly darker.	Little change.	Little change.	Little change.
18	*398	Yellowish brown.	Yellowish green.	Yellowish.	Brownish yellow.	No change.	No change.	No change.
19	605	Green.	Red.	Little change.	Dark green.	Little change.	Little change.	Little change.
20	604	Green.	Red.	Dull brown.	Dark green.	Little change.	Little change.	Little change.
21	188	Greenish black.	Bluish black.	Greenish blue.	Olive green.	Black.	No change.	No change.
22	***14	Orange yellow.	Orange yellow.	Little change.	Orange.	Dull brownish red.	No change.	No change.
23	21	Red.	Violet red.	Orange red.	Brownish red.	Brownish red.	Little change.	Little change.
24	318	Bluish green.	Blue.	Little change.	Greenish blue.	Red.	Redder.	Redder.
25	20	Red.	Red.	Little change.	Darker.	Reddish brown.	Brown.	Brown.
26	93	Violet.	Violet red.	Little change.	Violet.	Slightly bluer.	Orange red.	Orange red.
27	***480	Brown.	Blue.	Paler.	Brown.	Pale reddish.	Almost decolorized.	Almost decolorized.
28	*53	Violet red.	Scarlet.	Darker.	Violet red.	Brownish yellow.	No change.	No change.
29	*55	Red.	Scarlet.	Little change.	Little change.	Brownish yellow.	No change.	No change.
30	105	Violet.	Red.	Slightly bluer.	Reddish violet.	Dull brownish red.	Almost unchanged.	Almost unchanged.
31	4	Pale yellow.	Yellow.	Nearly decolorized.	Very pale dull brown.	No change.	No change.	No change.
32	***706	Red orange.	Dull orange red.	Little change.	Little change.	Violet red.	Violet red.	Violet red.

33	56	Red.	Little change.	Little change.	Dull orange.	Little change.
34	*62	Blue.	Darker.	Violet.	Dull brown.	Little change.
35	**64	Violet.	Violet red.	Violet.	Dull brown.	Little change.
36	*65	Blue.	Violet.	Blue.	Brown.	Little change.
37	**103	Violet.	Little change.	Violet.	Scarlet.	Scarlet.
38	139	Violet.		Dark violet.	Darker.	Little change.
39	164	Blue.				
40	**67	Orange red.	Slightly darker.	Brownish yellow.	Slightly paler.	Little change.
41	*169	Blue.	Blue.	Blue.	Dull violet red.	Little change.
42	163	Green.	Violet blue.	Green.	Dull violet.	Little change.
43	170	Blue.				
44	84	Yellow.	Orange.	Orange.	Dull orange red.	No change.
45	146	Reddish violet.	Blue.	Violet.	Brown.	No change.
46	287	Deep blue.	Darker.	Greenish blue.	Red.	Violet.
47	78	Violet red.	Brown.	Violet red.	Little change.	Little change.
48	**710	Violet.	Red.	Brown, dull.	Violet.	Violet.

TABLE 2.—*Behavior of dry colors or of dyed fibers with reagents—Continued.*

No.	Coloring matter.	Color of sulphuric acid solution	Color of dyed wool.	Reactions of dyed wool with reagents.			
				Concentrated hydrochloric acid.	Concentrated sulphuric acid.	10 per cent sodium hydroxid solution.	Ammonia solution, specific gravity, 0.95.
49	*546	Orange.	Orange yellow.	Yellow.	Orange.	Violet.	Violet.
50	1	Almost colorless.	Yellow.	Decolorized.	Pale brownish.	Little change (slightly darker).	No change.
51	507	Orange.	Reddish violet.	Slightly yellower.	Orange.	Red.	Red.
52	328	Reddish violet.	Orange yellow.	Dull violet.	Brownish violet.	Orange red.	Orange red.
53	606	Dull green.	Orange.	Yellowish brown.	Dark green.	Little change.	Little change.
54	154	Violet blue.	Red.	Blue.	Blue.	Brownish violet.	Little change.
55	85	Violet.	Orange.	Violet.	Violet.	Red, dark.	Red, dark.
56	**13	Orange yellow.	Orange.	Orange red.	Orange.	Slightly darker.	No change.
57	***86	Red.	Orange.	Red.	Red.	Dull orange red.	No change.
58	97	Red.	Orange.	Red.	Red.	Browner.	Browner.
59	54	Orange red.	Orange.	Scarlet.	Scarlet.	Little change.	No change.
60	329	Reddish violet.	Yellow.	Blue.	Reddish violet.	Orange yellow.	No change.
61	137	Brown.	Brown.		Darker.	Darker.	
62	157	Bluish green.	Red.	Blue violet.	Green.	Brownish violet.	Little change.
63	*95	Violet.	Orange yellow.	Violet red.	Violet.	No change.	No change.
64	88	Violet.	Orange yellow.	Violet red.	Violet.	No change.	No change.

65	*92	Violet red.	Yellow.	Violet red.	Violet red.	Dull brown.	Little change.
66	101	Blue.	Brown.	Reddish blue.	Blue.	Violet red.	Violet red.
67	102	Violet.	Red.	Red.	Violet.	Dull orange red.	No change.
68	483	Brownish yellow.	Orange red.	Orange yellow.	Dull orange yellow.	Little change.	Little change.
69	**510	Yellow.	Yellow.	Little change.	Little change.	Slightly redder, green fluor.	Little change, green fluor.
70	26	Orange yellow.	Orange yellow.	Orange.	Orange.	Orange red.	Orange.
71	220	Reddish violet.	Yellow.	Brown.	Violet red.	Red orange.	Orange yellow.
72	269	Reddish violet.	Yellow.	Brown.	Violet red.	Red orange.	Orange yellow.
73	512	Yellow.	Red.	Yellow orange.	Yellow orange.	No change.	No change.
74	515	Brownish yellow.	Violet red.	Yellow.	Brownish.	Scarlet.	Little change.
75	516	Brownish yellow.	Orange red.	Orange yellow.	Orange yellow.	No change.	No change.
76	517	Brownish yellow.	Violet red.	Orange yellow.	Orange yellow.	No change.	No change.
77	518	Brownish yellow.	Violet red.	Yellow.	Orange.	No change.	No change.
78	520	Orange.	Violet red.	Yellowish.	Orange.	No change.	No change.
79	521	Orange.	Violet red.	Yellowish.	Orange.	No change.	No change.
80	*523	Orange.	Red violet.	Almost decolorized.	Orange.	No change.	No change.

TABLE 2.—*Behavior of dry colors or of dyed fibers with reagents—Continued.*

No.	Color of sulphuric acid solution.	Color of dyed wool.	Reactions of dyed wool with reagents.				Ammonia solution, specific gravity, 0.95.
			Concentrated hydrochloric acid.	Concentrated sulphuric acid.	10 per cent sodium hydroxid solution.		
81	2	Pale yellow.	Orange yellow.	Almost decolorized.	Pale brownish yellow.	No change.	No change.
82	*3	Pale yellow.	Yellow.	Almost decolorized.	Pale brownish yellow.	Little change.	Little change.
83	6	Very pale yellow.	Orange.	Almost decolorized.	Pale brownish yellow.	Dull orange red.	Slightly redder.
84	5¾	Orange red.	Orange.	Yellow.	Redder.	Violet.	Violet.
85	***707	Orange.	Yellow.	Red.	Reddish brown.	Orange.	Orange.
86	*10	Yellowish orange.	(Silk.) Orange yellow.	(Silk.) Orange yellow.	(Silk.) Brownish yellow.	(Silk.) Orange yellow.	(Silk.) No change.

87	*468	Orange yellow.	Violet.	Pale orange yellow.	Pale dull orange.	Decolorized.	Decolorized.
88	464	Yellow.	Bluish violet.	Pale orange yellow.	Pale dull orange.	Decolorized.	Decolorized.
89	438	Yellowish brown.	Green.	Orange yellow.	Brownish yellow.	Decolorized.	Paler.
90	**423	Yellow.	Green.	Pale orange yellow.	Pale dull yellow.	Decolorized.	Decolorized.
91	442	Yellow.	Greenish blue.	Pale orange yellow.	Pale dull yellow.	Little change.	Little change.
92	476	Reddish brown.	Blue.	Little change.	Reddish brown.	Dull brown.	Little change.
93	240	Blue.	Reddish brown.	Blue.	Blue.	No change.	No change.
94	277	Blue.	Brownish red.	Blue.	Greenish blue.	Orange red.	Orange red.
95	562	Brownish red.	Violet blue.	Orange red.	Brownish red.	Green.	Blue.
96	658	Colorless.	Yellow.	Decolorized.	Almost decolorized.	Decolorized.	Paler.
97	496	Orange.	Red.	Orange yellow.	Yellow.	Paler.	Paler.
98	650	Yellowish green.	Blue.	Almost decolorized.	Greenish, paler.	Pale violet.	No change.
99	639	Dark green.	Blue.	Violet blue.	Dull greenish.	Pale dull violet red.	Pale dull violet red.
100	*554	Green.	Red.	Greenish blue.	Green.	Red.	Red.
101	**448	Yellowish brown.	Violet red.	Yellowish brown.	Dull brown.	Decolorized.	Paler.

TABLE 2—*Behavior of dry colors or of dyed fibers with reagents—Continued.*

No.	Coloring matter.	Color of sulphuric acid solution.	Color of dyed wool.	Reactions of dyed wool with reagents.			
				Concentrated hydrochloric acid.	Concentrated sulphuric acid.	10 per cent sodium hydroxide solution.	Ammonia solution, specific gravity, 0.95.
102	***425	Colorless.	Yellow.	Decolorized.	Nearly decolorized.	Decolorized.	Paler.
103	426	Colorless.	Yellow.	Decolorized.	Nearly decolorized.	Decolorized.	Paler.
104	***451	Yellow.	Violet.	Yellowish.	Yellowish.	Decolorized.	Almost decolorized.
105	452	Yellow.	Violet.	Yellowish.	Yellowish.	Decolorized.	Almost decolorized.
106	**427	Yellow.	Bluish green.	Almost decolorized.	Almost decolorized.	Decolorized.	Decolorized.
107	428	Yellow.	Green.	Almost decolorized.	Almost decolorized.	Decolorized.	Decolorized.
108	*197	Brown.	Reddish brown.	Redder, darker.	Browner.	Yellow.	Yellow.
109	201	Brown.	Reddish brown.	Redder, darker.	Browner.	Yellow.	Yellow.
110	17	Orange yellow.	Orange yellow.	Orange red.	Brownish red.	Slightly duller.	Yellow.
111	18	Orange yellow.	Orange yellow.	Red.	Brown.	Slightly duller.	Yellow.
112	505	Yellow.	Violet red.	Orange.	Yellow.	Slightly bluer.	Slightly bluer.
113	499	Yellow.	Bluish red.	Orange.	Yellow, pale.	Slightly bluer.	Slightly bluer.
114	***594	Yellowish brown.	Bluish red.	Orange.	Yellow.	Bluer.	Bluer.
115	502	Yellowish brown.	Bluish red.	Orange.	Yellow.	Slightly bluer.	Slightly bluer.

No.	Coloring matter.	Color of sulphuric acid solution.	Color of dyed silk.	Reactions of dyed silk with reagents.		
				Concentrated hydrochloric acid.	Concentrated sulphuric acid.	10 per cent sodium hydroxide solution.
116	**16. Dimethylaminoazobenzene.	Yellow.	Orange yellow.	Red, bluish.	Orange yellow.	No change.
117	7. Aminoazobenzene.	Yellow.	Orange yellow.	Brownish red.	Orange yellow.	Little change.
118	*. Ortho-aminoazotoluene.	Orange yellow.	Orange yellow.	Dull orange.	Orange yellow.	Little change.
119	Benzeneazo- β -naphthylamin.	Reddish violet.	Yellowish brown.	Red.	Violet.	Little change.
120	Ortho-tolueneazo- β -naphthylamin.	Violet.	Yellowish brown.	Red.	Violet.	Little change.
121	666. Quinophthalon.	Reddish brown.	Yellow.	Orange yellow.	Brownish yellow.	Orange yellow.
122	Benzeneazo- α -naphthol.	Violet.	Orange.	Violet red.	Violet.	Deep red.
123	59. Naphthaleneazo- α -naphthol.	Greenish blue.	Brown.	Bluish violet.	Greenish blue.	Bluish red.
124	Ortho-tolueneazo- α -toluene.	Green.	Red.	Blue.	Green.	Reddish blue.
125	*11. Benzeneazo- β -naphthol.	Red.	Orange.	Orange red.	Red.	Little change.
126	**49. Meta-xyleneazo- β -naphthol.	Violet red.	Orange red.	Red.	Violet red.	No change.
127	60. α Naphthaleneazo- β -naphthol.	Bluish violet.	Bluish red.	Slightly bluer.	Dull blue.	Browner and paler.
128	*143. Benzeneazobenzeneazo- β -naphthol.	Green.	Red.	Violet, then brown.	Green.	Violet red.
129	*. Ortho-tolueneazo- α -tolueneazo- β -naphthol.	Green.	Violet red.	Violet blue.	Bluish green.	Little change.
130	31. Para-nitrobenzeneazo- β -naphthol.	Violet red.	Brownish red.	Orange red.	Violet red.	Little change.
						No change.

With concentrated solution of stannous chlorid in hydrochloric acid the fiber is decolorized in all cases except that of No. 666.

TABLE 3.—*Behavior of colors when treated with reducing agents followed by oxidizing agents.*

[Numbers denoting permitted dyes are in boldfaced type; natural colors are in italics. Dyes common in foods are starred, three stars indicating those most often found. Statements apply in general to 0.01 per cent solutions.]

No.	Coloring matter.	With sodium hydrosulphite.	Coloring matter.	Reduction product with air or potassium persulphate.
1	*462	Almost decolorized.	*462	Color restored.
2	434	Almost decolorized.	434	Color partially restored.
3	435	Almost decolorized.	435	Color partially restored.
4	*436	Becomes paler very slowly.	*436	
5	439	Paler, slowly.	439	Color restored.
6	491	Almost decolorized.	491	Some color returns.
7	440	Decolorized.	440	Color restored.
8	**602	Pale olive.	**602	Color restored.
9	**108	Decolorized.	**108	Remains colorless or nearly so.
10	***8	Decolorized.	***8	Remains colorless or nearly so.
11	9	Decolorized.	9	Remains colorless or nearly so.
12	**89	Decolorized.	**89	Remains colorless or nearly so.
13	692	Decolorized.	692	Color restored.
14	399	Not decolorized.	399	
15	***106	Decolorized.	***106	Remains colorless or nearly so.
16	107	Decolorized.	107	Remains colorless or nearly so.
17	94	Decolorized.	94	Remains colorless or nearly so.
18	*398	Decolorized.	*398	Remains colorless or nearly so.
19	605	Pale orange.	605	Color restored.
20	604	Pale orange.	604	Color restored.
21	198	Decolorized.	198	Remains colorless or nearly so.
22	**14	Decolorized.	**14	Remains colorless or nearly so.
23	21	Decolorized rather slowly.	21	Remains colorless or pale brownish.
24	318	Bluer; then decolorized.	318	Remains colorless or nearly so.
25	20	Decolorized.	20	Remains colorless or nearly so.
26	93	Decolorized.	93	Remains colorless or nearly so.
27	***480	Much paler.	***480	Color restored.
28	*53	Decolorized.	*53	Remains colorless or nearly so.
29	*55	Decolorized.	*55	Remains colorless or nearly so.
30	105	Decolorized.	105	Remains colorless or nearly so.
31	4	Decolorized.	4	Remains colorless or nearly so.
32	***706	Not decolorized.	***706	
33	56	Decolorized.	56	Remains colorless or nearly so.
34	*62	Decolorized.	*62	Remains colorless or nearly so.
35	**64	Decolorized.	**64	Remains colorless or nearly so.
36	*65	Decolorized.	*65	Remains colorless or nearly so.
37	*103	Decolorized.	*103	Remains colorless or nearly so.
38	139	Decolorized.	139	Remains colorless or nearly so.
39	164	Decolorized.	164	Remains colorless or nearly so.
40	*667	Not changed.	*667	
41	*169	Bluer; then decolorized.	*169	Remains colorless or nearly so.
42	163	Decolorized.	163	Remains colorless or nearly so.
43	170	Bluer; then decolorized.	170	Remains colorless or nearly so.
44	84	Decolorized.	84	Remains colorless or nearly so.
45	146	Bluer; then decolorized.	146	Remains colorless or nearly so.
46	287	Slowly decolorized.	287	Remains colorless or nearly so.
47	78	Slowly decolorized.	78	Remains colorless or nearly so.
48	***710	Decolorized, nearly.	***710	Color restored.
49	*546	Not changed.	*546	
50	1	Decolorized.	1	Not restored.
51	507	Not changed.	507	
52	328	Decolorized.	328	Remains colorless or nearly so.
53	606	Pale yellow.	606	Color restored.
54	154	Bluer; then decolorized.	154	Remains colorless or nearly so.
55	85	Decolorized.	85	Remains colorless or nearly so.
56	*113	Decolorized.	*113	Remains colorless or nearly so.
57	***86	Decolorized.	***86	Remains colorless or nearly so.
58	97	Decolorized.	97	Remains colorless or nearly so.
59	54	Decolorized.	54	Remains colorless or nearly so.
60	329	Decolorized.	329	Remains colorless or nearly so.
61	139	Decolorized.	139	Remains colorless or nearly so.
62	157	Decolorized.	157	Remains colorless or nearly so.
63	*95	Decolorized.	*95	Colorless or slightly yellow.
64	88	Decolorized.	88	Colorless or slightly yellow.
65	*92	Decolorized.	*92	Remains colorless or nearly so.
66	101	Decolorized.	101	Remains colorless or nearly so.
67	102	Decolorized.	102	Remains colorless or nearly so.
68	483	Decolorized.	483	Color restored.
69	*510	Much paler.	*510	Color restored.
70	26	Decolorized.	26	Remains colorless or nearly so.
71	220	Decolorized.	220	Remains colorless or nearly so.
72	269	Decolorized.	269	Remains colorless or nearly so.
73	512	Much paler (with excess).	512	Color restored.
74	515	Much paler (with excess).	515	Color restored.
75	516	Much paler (with excess).	516	Color restored.

TABLE 3.—*Behavior of colors when treated with reducing agents followed by oxidizing agents—Continued.*

No.	Coloring matter.	With sodium hydrosulphite.	Coloring matter.	Reduction product with air or potassium persulphate.
76	517	Much paler (with excess).	517	Color restored.
77	518	Much paler (with excess).	518	Color restored.
78	520	Much paler (with excess).	520	Color restored.
79	521	Much paler (with excess).	521	Color restored.
80	*523	Much paler (with excess).	*523	Color restored.
81	2	Decolorized.	2	Remains colorless or nearly so.
82	*3	Decolorized.	*3	Remains colorless or nearly so.
83	6	Dark; then pale.	6	Pale reddish.
84	534	(Alk. sol.), red, slowly.	534	Color restored.
85	***707	Not reduced.	***707	
86	*10	Decolorized.	*10	Remains colorless or nearly so.
87	*468	Decolorized.	*468	Color restored.
88	464	Decolorized.	464	Color restored.
89	438	Almost decolorized.	438	Color restored.
90	***433	Paler.	***433	Greener.
91	442	Paler, slowly.	442	Restored.
92	476	Not readily reduced.	476	
93	240	Almost decolorized.	240	Remains colorless or nearly so.
94	277	Browner; then colorless.	277	Remains colorless or nearly so.
95	562	(Alk. sol.), yellow.	562	Color restored.
96	658	No change.	658	
97	496	Almost decolorized.	496	Color largely restored.
98	650	Decolorized.	650	Color restored.
99	639	Decolorized.	639	Color restored.
100	*584	Decolorized.	*584	Color restored.
101	***448	Decolorized.	***448	Color restored.
102	***425	Not decolorized.	***425	
103	426	Not decolorized.	426	
104	***451	Decolorized.	***451	Color restored.
105	452	Decolorized.	452	Color restored.
106	*427	Decolorized.	*427	Color restored.
107	428	Decolorized.	428	Color restored.
108	*197	Almost decolorized.	*197	Colorless or nearly so.
109	*201	Almost decolorized.	*201	Colorless or nearly so.
110	17	Decolorized.	17	Remains colorless or nearly so.
111	18	Decolorized.	18	Remains colorless or nearly so.
112	505	Not decolorized.	505	
113	499	Not decolorized.	499	
114	***504	Not decolorized.	***504	
115	502	Not decolorized.	502	

OXIDATION WITH BROMIN.

This test is valuable for quickly testing the color solutions obtained in the fractionation. The free acid need not be removed; though, as described in detail below, minor differences exist, depending on whether the solutions are practically neutral or markedly acid. They must not be alkaline and should be free from foreign material, though dissolved amyl alcohol, etc., does not interfere. With the oil-soluble dyes, the oxidation should be made in acetic acid of from 50 to 80 per cent strength.

The chief practical use of the test is for the detection of the azo and the azin dyes, especially when in admixture with natural coloring matters. It provides the simplest means for the identification of the "first component" of the azo colors, for which, of course, well-known reduction methods may also be applied. The test is made as follows: About 5 cc of the dye solution (preferably of concentration in the neighborhood of from 0.005 to 0.01 per cent) are treated with bromin water (1 per cent) added drop by drop until a little more has been used than is required to destroy the dye. A few drops of 3 per cent hydrazin sulphate solution are then added and the mix-

ture divided into two portions. To one is first added a few drops of alcoholic alpha-naphthol solution, then excess of sodium carbonate; to the other, sodium carbonate only. With azo compounds sodium formate may be substituted for the hydrazin salt.

The reactions obtained are referred to classes as follows:

Class A—Azo dyes.—These yield on oxidation in acid or neutral solutions a diazo compound corresponding to the "first component" of the dye.¹ The azo group remains attached to the nonhydroxylated or nonaminated residue, and it is noteworthy that with Chrysophenin (No. 329), the one azo color described in the table containing neither hydroxyl nor amino groups, the usual reaction is not obtained. With dis-azo colors of the type of cotton scarlet, $C_6H_5N_2-C_6H_4N_2C_{10}H_5OH(SO_3Na)_2$, the azo group between the two nonhydroxyl-containing residues is not readily attacked, so that a diazo-azo compound is formed. With dyes of class A the solution becomes colorless, pale yellow, or pale orange on addition of bromin. After adding the hydrazin sulphate the solution is colorless or pale brownish or pinkish, a tendency to show a slight coloration being more marked, the more nearly neutral the solution. Addition of sodium carbonate alone produces no marked coloration, but alpha-naphthol, followed by the carbonate, gives a pronounced color. It is advisable to add some ether to the colored mixture and shake; since if the first component of the original dye was an unsulphonated amin (indicated by "e" in the table) the new coloring matter formed will be taken up by ether from the alkaline mixture, giving usually an orange solution that on being poured off and treated with a large excess of concentrated hydrochloric acid becomes, in most cases, violet or blue. If the new dye is sulphonated (indicated by "w"), it will not be extracted by the ether from the mixture. When desired, the alpha-naphthol derivative, after separation by a suitable solvent, may be dyed on wool or silk and further identified by the ordinary spot tests with acids and alkalies. See tables for solubilities and reactions of Orange I (No. 85), Fast brown N (No. 101), benzeneazo- α -naphthol, and α -naphthaleneazo- α -naphthol (serial numbers in tables: 55, 66, 122, 123, respectively).

Other compounds, such as α -naphthylamin, readily coupling with diazo compounds, may, of course, be used instead of the α -naphthol, sodium acetate being substituted for the sodium carbonate when an amin is employed. With the simple monazo colors, the reaction seems almost quantitative. With benzidin dyes it takes place least smoothly.

Class AA—Azo dyes, reacting like the preceding class in solutions that contain a considerable amount of free hydrochloric acid (perhaps one-half normal or above).—In neutral solutions other oxidation

¹ For action of halogens on azo compounds, see M. Schmidt, J. prakt. Chem. **84** (1912), 235. Oxidation by lead peroxid, Lauth, Bul. Soc. Chim. **6** (1891) III, 94; by nitric acid, O. Schmidt, Ber. Chem. Ges. **38** (1905), 3201, 4022. See also Meldola, Proc. Chem. Soc. **10** (1894), 118, and Trans. Chem. Soc. **55** (1889), 608, and **65** (1894), 841.

reactions take place, so that a more or less strong coloration is produced on treatment with sodium carbonate without previous addition of the naphthol. Crystal Scarlet (No. 64), Bordeaux B (No. 65), and Palatine Red (No. 62) give strong blue colorations; Naphthol Black (No. 88) and Amaranth (No. 107) a less intense blue color. Azorubin (No. 103) gives a purple changing to bluish red, but not very intense. Except for the first three dyes named, the colorations are considerably less intense than the original dyes, and the reaction much less trustworthy and valuable than the smooth, almost quantitative, reaction in acid solutions. When treated with bromin in solutions containing sodium carbonate (one-fourth normal), Nos. 62, 64, and 65 are bleached, becoming intensely blue on addition of hydrazin sulphate.

Class B.—Azin derivatives,¹ etc.—On treatment with bromin in neutral or acid solutions, the color is readily bleached, but is restored on adding hydrazin sulphate. Sodium carbonate and alpha-naphthol plus sodium carbonate produce no change other than that shown by the original dye solution on treatment with alkali. A few dyes of Classes A and AA when oxidized in neutral solutions tend to show a rather marked coloration on adding the hydrazin sulphate. However, with the typical members of Class B, the dye may be bleached and restored a number of times by careful alternate addition of the two reagents, the bromin apparently forming a nearly colorless compound reconverted into the original dye on addition of the hydrazin sulphate.

Class C.—Colors giving precipitates at dilutions as high as 0.01 per cent.—This class includes most of the basic dyes.

Class D.—Colors showing marked changes in tint in neutral or very faintly acid solution on addition of bromin.²—The colorations are usually produced by a mere trace of the halogen and destroyed by excess, and the reactions are consequently not very dependable or valuable. With many of the dyes of this type containing alkylated amino groups the color change would seem to be due to elimination of alkyl radicals. In general with dyes of this group the coloring matter, while readily altered, is completely broken down by bromin only with difficulty. On addition of hydrazin sulphate no change takes place other than that due to removal of the excess of the colored halogen.³ The coloration with alpha-naphthol and sodium carbonate is identical with that produced by sodium carbonate alone. With most of the yellow coloring matters of this group it is

¹ Quinophthalon by treatment with bromin first forms the unstable colorless addition product containing two atoms of bromin in the molecule (Eibner and Lange, *Liebigs Annalen der Chemie*, **315** (1901), 315). For action on azin dyes compare Vaubel, *J. prakt. Chem.*, **54** (1896), 289.

² According to Heumann, *Die Anilinfarben*, vol. 1, p. 41, Malachite Green solution, on oxidation with lead peroxid and acetic acid, becomes violet, and then contains the salt of diaminotriphenylcarbinol. Compare further Vaubel, *J. prakt. Chem.*, **50** (1894), 347.

³ Chlorin water may be used if preferred.

brownish. With most of the others it is ill-defined and is probably often produced by a small fraction of the dye that has escaped destruction by bromin. In acid solution these dyes are merely destroyed by bromin in most cases, the results being as given for E.

Class E.—Dyes in this class are similar to those in Class D, but show no color changes other than bleaching, on addition of bromin.

Class F.—*Halogenated fluorescein derivatives and similar compounds.*—These dyes are very resistant to bromin. The nonfluorescent iodine compounds tend to become yellower in shade and to develop a green fluorescence, probably due to partial substitution of iodine by bromine.

TABLE 4.—*The bromine test: Classification of colors according to reactions obtained.*

[Numbers denoting permitted dyes are in boldfaced type; natural colors are in italics. Dyes common in foods are starred, three stars indicating those most often found. Statements apply in general to 0.01 per cent solutions.]

No.	Coloring matter.	Class.	No.	Coloring matter.	Class.	No.	Coloring matter.	Class.	No.	Coloring matter.	Class.
1	*462	D or E.	33	56	A (e).	65	*92	A (w).	87	*468	E.
2	434	D.	34	*62	AA (e).	66	101	A or AA (w).	88	464	E.
3	435	D.	35	*64	AA (e).				89	438	D.
4	*436	D.	36	*65	AA (e).	67	102	A (w).	90	*433	D.
5	439	E.	37	*103	A or AA (w).	68	483	E.	91	442	E.
6	491	D or E.				69	*510	A (3).	92	476	E.
7	440	E.	38	139	A or AA (w).	70	26	A (e).	93	240	A. ⁵
8	*602	E (B). ¹				71	220	A. ¹	94	277	A.
9	*108	A (w).	39	164	A (w).	72	269	A. ¹	95	562	B.
10	*88	A (w).	40	*667	B.	73	512	E.	96	658	E.
11	9	A (w).	41	*169	A (w).	74	515	E.	97	496	D or C.
12	*89	A (w).	42	163	A (w).	75	516	E.	98	650	E or C.
13	692	E.	43	170	A (w).	76	517	E.	99	639	E or D.
14	399	A (w).	44	84	A (w).	77	518	E.	100	*584	B or C.
15	*106	A (w).	45	146	A (e).	78	520	E.	101	*448	E or D.
16	107	AA or A (w).	46	287	A. ¹	79	521	E.	102	*425	E or C.
			47	78	A. ²	80	*523	E.	103	426	E or C.
17	94	A (w).	48	*110	E.	81	2	E.	104	*451	E or C.
18	*398	E.	49	*546	B. ¹	82	*3	E.	105	452	E or C.
19	605	B.	50	1	E.	83	6	E.	106	*427	D or C.
20	604	B.	51	507	D.	84	534	B.	107	428	D or C.
21	188	AA (w).	52	328	A (w).	85	*707	E.	108	*197	A or C.
22	*14	A (e).	53	606	B.	86	*10	A (e). ⁴	109	*201	A or C.
23	21	A (e).	54	154	A (e).				110	17	A or C (e).
24	318	A. ¹	55	85	A (w).				111	18	A or C (e).
25	20	A (e).	56	*13	A (e).				112	505	D or C.
26	93	A or AA (w).	57	*86	A (w).				113	499	D or C.
			58	97	A (w).				114	*504	D or C.
27	*480	D.	59	54	A (e).				115	502	D or C.
28	*53	A (e).	60	329	E.						
29	*55	A (e).	61	139	A (w).						
30	105	A (w).	62	157	A (e).						
31	4	E.	63	*95	A (w).						
32	*706	E.	64	88	A (w).						

¹ Imperfectly.

² Some alcohol should be added before the alpha-naphthol.

³ Gives eosin.

⁴ Of the oil-soluble dyes given in the other tables all belong to type A except Quinophthalon, which in 60 per cent acetic acid shows reaction indicated under B.

⁵ Very imperfectly.

REACTIONS WITH NITROUS ACID.

By treatment with nitrous acid in dilute solution most of the common coal-tar dyes used in food coloring are not readily affected. A considerable number, however, show marked changes, because of diazotization of free amino groups, of formation of nitroso compounds, or of direct oxidation.

Where diazo compounds are formed, they may be further coupled by the usual method of adding the mixture to an alkaline solution of one of the naphthols, or of a naphthol sulphonic acid. B. C. Hesse has pointed out that the two acid yellows (No. 8 and No. 9) can be distinguished by the use of alpha-naphthol—No. 9 giving in alkaline solution a red compound; No. 8 one which is intensely blue.

In the test described below the mixture is treated first with nitrous acid, then with hydrazin sulphate. The hydrazin sulphate serves to destroy the excess of nitrous acid, so that the naphthol solution (or an amin if preferred) may be added directly, and the coupling then brought about by addition of alkali. The new dye formed may also be separated readily, if desired, by acidifying and shaking out with a suitable solvent. In the case of only one dye in the table, Safranin (No. 584), does the diazo compound appear to be reduced or changed by addition of hydrazin sulphate.

The nitroso compounds formed from Nos. 95 and 88, are decomposed by the hydrazin salt, the original color of the acid solution being restored.

The test is carried out as follows: The solution of the color at ordinary temperature is made slightly acid by the addition of two or three drops of concentrated hydrochloric acid and one or two drops of 7 per cent sodium nitrite solution are added. With blue or green dyes, where oxidation changes may take place, the mixture may be allowed to stand a few minutes at this stage; but with other colors about 1 cc, or an excess, of 3 per cent hydrazin sulphate solution is added at once. The mixture is allowed to stand one-half or one minute to permit complete destruction of the excess of nitrous acid; then it is divided and a few drops of alpha-naphthol solution are added to one portion. Both portions are then made strongly alkaline with sodium carbonate, the one not containing alpha-naphthol serving as a check to show if any coupling has taken place.

TABLE 5.—*Behavior of colors when treated with sodium nitrite.*

[° Indicates that no color changes take place other than those produced by the acid or alkali.]

462.—With sodium nitrite, blue; then colorless; after making alkaline in the presence of alpha-naphthol, orange.

434°, 435°, 436°.—Attacked very slowly by nitrous acid.

439.—Becomes yellow with sodium nitrite.

491.—Becomes violet with sodium nitrite (rather slowly).

440°, 602°, 108°.

8.—With sodium nitrite, much paler; after adding alpha-naphthol and excess of sodium carbonate, intensely blue.

9.—With sodium nitrite, much paler; after adding alpha-naphthol and excess of sodium carbonate, red.

89.—Red solution becomes yellow with sodium nitrite; on addition of hydrazin sulphate, red again..

692.—With sodium nitrite, slowly oxidized to the yellow isatin derivative.

399°, 106°, 107°, 94°.

398.—With sodium nitrite, brown.

605°, 604°, 188°, 14°.

21.—With sodium nitrite, slightly darker; with alpha-naphthol and sodium carbonate, dull greenish black.

318.—With sodium nitrite, paler and redder.

20°, 93° (480°.—Slowly attacked by nitrous acid); 53°, 55°, 105°, 4°, 706°, 56°, 62°, 64°, 65°, 103°, 139°, 164°, 667°, 169°, 163°, 170°.

84.—With sodium nitrite, redder.

146°, 287°, 78°, 710°, 546°, 1°.

507.—With sodium nitrite, bluer.

328°, 606°, 154°.

85.—With sodium nitrite, paler.

86°, 54°, 13°, 97°, 329°, 139°, 157°.

95.—Crimson solution becomes yellow with sodium nitrite; on addition of hydrazin sulphate, red again.

88.—Crimson solution becomes yellow with sodium nitrite; on addition of hydrazin sulphate, red again.

92°.

101.—Paler with sodium nitrite.

102°, 483°, 510°, 26°.

220, 229.—Slightly paler with sodium nitrite.

512°, 515°, 516°, 517°, 518°, 520°, 521°, 523°, 2°, 3°, 6°, 534°, 707°, 10°, 468°, 464°, 438°, 433°, 442°, 476°, 240°, 277° (562°, scarcely attacked; in 50 per cent acetic acid, behaves with nitrous acid as with bromin in the bromin test); 658°, 496°, 650°, 639°.

584.—With sodium nitrite, blue; rather rapidly becomes red again on addition of hydrazin sulphate.

448.—Wine-red on diazotization, addition of hydrazin sulphate, alpha-naphthol and sodium carbonate; with sodium nitrite in acetic acid solution, first blue, then colorless.

425°, 426°, 451°, 452°.

427.—Reddish with sodium nitrite.

197°, 201°.

17.—With sodium nitrite, paler; after addition of sodium carbonate, naphthol, etc., somewhat redder.

18.—With sodium nitrite, paler; after addition of sodium carbonate, naphthol, etc., somewhat redder.

505°, 499°, 504°, 502°.—May appear bluer when the alcoholic alpha-naphthol solution is added.

16°.—Slowly destroyed by nitrous acid.

7.—Paler with sodium nitrite; after addition of other reagents, red.

Aminoazotoluene.—As stated above for 7.

Benzeneazo- β -naphthylamin, Ortho-tolueneazo- β -naphthylamin.—These compounds are almost insoluble in aqueous liquids. As ortho-aminoazo derivatives, they are not readily diazotized or coupled.

REACTIONS WITH POTASSIUM CYANID.

With the common monazo dyes, the bromin oxidation will provide for an identification of the "first component" of the color, i. e., the radical not containing the hydroxyl or amino groups. The other radical, usually containing hydroxyl or amino groups ortho to the azo junction, is identified with much more difficulty in most cases. Since the two ortho-azo dyes permitted in foods are both derived from 2-naphthol-3-6-disulphonic acid as second component, the reaction discovered by Lange¹ that derivatives of this acid are attacked on boiling with potassium cyanid and the 3-sulphonic acid group replaced by cyanogen, is useful for distinguishing and separating isomeric dyes.

The test may be made as follows: About 10 cc of the neutral color solution is treated with 1 cc of 20 per cent potassium cyanid solution

¹ Deutsches Reichs Patent No. 189,035.

and 1 cc of 20 per cent ammonium chlorid solution, and is heated in a test tube in a boiling water bath for from five to eight minutes. It is then quickly cooled. The reactions obtained with certain dyes are shown in the table. The test requires some care, and blanks with known dyes should be carried through at the same time in all cases.

The results with a number of common azo dyes are shown in Table 6, the derivatives of 2-naphthol-3-6-disulphonic acid forming new dyes of markedly different solubilities, corresponding to the fact that they contain one less sulphonic acid group. By warming with the cyanid solution for a considerable period of time further reactions easily take place, derivatives of 2-naphthol-3-6-disulphonic acid and 2-naphthol-6-8-disulphonic acid being especially unstable.

The common nitro dyes are changed by warming with cyanid solution, becoming brownish or reddish (compare formation of isopurpuric acid from trinitrophenol).

TABLE 6.—*Behavior of colors with cyanid solution.*

Dye.	"Second component" of dye.	Behavior with cyanid solution.
Azorubin S. G.	108 Naphthol trisulphonic acid (2-3-6-8).	Warmed 8 minutes, dye almost completely destroyed with production of orange and yellow substances. Warmed until dark red (1-2 minutes), strongly acidified, and washed with 2 N HCl, practically no color is removed (3-4 washings); then washed with N/4 HCl, a bluish red dye is readily removed. Apparently unchanged by cyanid.
	106 Naphthol disulphonic acid (2-6-8).	Dye is not changed in solubility, although on long warming much color is destroyed. The cyanid mixture may be acidified with 5 cc concentrated hydrochloric acid, and shaken out with 10 cc of amyl alcohol. On separating the alcohol, and washing 4 or 5 times with fourth-normal hydrochloric acid, nearly all of the dye will be taken out by the dilute acid.
	107 Naphthol disulphonic acid (2-3-6).	Dye is changed into a cyan-derivative similar in solubility to other disulphonated monazo dyes. The cyanid mixture is pale brown and when treated as stated under New Coccin (106), almost all coloring matter remains in the amyl alcohol. On long heating of the cyanid mixture the cyan-derivative may be completely destroyed, further reactions taking place.
	14 Naphthol disulphonic acid (2-6-8).	Dye unchanged. Cyanid mixture, when acidified with 1 cc glacial acetic acid and shaken with 5 to 10 cc amyl alcohol, gives up little coloring matter to the latter.
	15 Naphthol disulphonic acid (2-3-6).	Dye changed into a cyan-derivative similar in solubility to the other monosulphonated monazo dyes. The cyanid mixture is pale brownish, and when treated as described under Orange G (14) gives up most of its coloring matter to the alcohol.
	20 Dioxynaphthalene disulphonic acid (1-8-3-6).	As stated for 14.
	21 Aminonaphthol disulphonic acid (1-8-3-6).	As stated for 14.
	52 Naphthol disulphonic acid (1-4-8).	As stated for 14.
	53 Naphthol disulphonic acid (1-3-6).	As stated for 14.
	55 Naphthol disulphonic acid (2-3-6).	As stated for 15.
	56 Naphthol disulphonic acid (2-3-6).	As stated for 15.
	62 Naphthol disulphonic acid (1-3-6).	As stated for 14.
	64 Naphthol disulphonic acid (2-6-8).	As stated for 14.
	65 Naphthol disulphonic acid (2-3-6).	As stated for 15.

NATURAL COLORING SUBSTANCES.

Relatively few good tests are known for the common natural colors. For properties useful in analysis, see especially the tables given in United States Department of Agriculture, Bureau of Chemistry Circular No. 63. Some of the common properties considered most useful for the characterization of different colors are summarized below.

By addition of concentrated hydrochloric acid, the yellow ether or alcohol solutions of carotin and xanthophyll show little change, becoming perhaps slightly paler; green chlorophyll solutions become yellower or browner; annatto in ether or alcohol solution remains orange, not changing perceptibly with acid. Turmeric solutions in ether or alcohol show a pure yellow color with more or less green fluorescence, and on addition of several volumes of concentrated hydrochloric acid the color passes to orange red or carmine red. The orange or orange yellow solutions of logwood, also of the redwoods, barwood, sandalwood, camwood, and brazilwood, become deep red with excess of hydrochloric acid. The slightly colored neutral or faintly acid aqueous solutions of the flavone colors of fustic, Persian berries, quercitron, etc., become intensely yellow with from 2 to 4 volumes of concentrated acid. Neutral or slightly acid solutions of cochineal, archil, saffron, and caramel show little change.

The slightly acid solutions of the various coloring matters show the behavior described below, when treated with a little sodium hydroxide solution: Carotin and xanthophyll, little change; chlorophyll, "brown phase" reaction; alkanet, deep blue; turmeric, orange brown; the redwoods, violet red; logwood, violet to violet blue. The flavone colors become bright yellow; saffron remains yellow, showing little change. The red solutions of archil and the orange of cochineal become blue and violet, respectively. Caramel shows little change, becoming slightly deeper brown. The red fruit colors (in presence of air) become dull blue, green, or brown.

By sodium hydrosulphite in acid solution, the yellow coloring matters are little affected. Logwood is almost decolorized, the color returning imperfectly. Archil is decolorized, the color returning when shaken with air. The reaction is more easily seen in alkaline solution. Cochineal shows no marked change. The anthocyanidins derived by hydrolysis from the red fruit colors are almost decolorized by hydrosulphite. Caramel is rendered slightly paler.

In the bromin test all coloring matters, except alkanet, are merely destroyed more or less completely by the halogen, hence they belong in general to Class E. The flavone colors tend to become darker with the first addition of bromin. Alkanet (best in alcoholic solution) corresponds to Class B.

Ferric chlorid gives no marked change with annatto, turmeric, or saffron, these perhaps, appearing somewhat browner. With the flavone colors, colorations varying from dark olive green to black are produced. With the redwoods and logwood, very dark shades of violet, brown, or black are obtained. Cochineal becomes somewhat darker. Caramel is not affected. The solutions must be practically neutral.

By addition of alum solution the yellow color of logwood is changed to rose red (rather slowly). The redwoods are affected similarly. The pale yellow solutions of the flavones become more strongly yellow, that of fustic developing a green fluorescence. Saffron and turmeric show little change.

Uranium acetate in neutral or nearly neutral solutions gives orange colorations with the flavones. Turmeric becomes somewhat browner; saffron is not affected; cochineal becomes green; alkanet, yellowish green to bluish green; logwood, violet, quickly fading.

The coloration with concentrated sulphuric acid dropped on the dry coloring matter is for carotin and xanthophyll, blue, usually obtained with difficulty. Annatto and saffron also give blue colors; turmeric, a red; the flavone colors, yellow or orange colorations; alkanet and archil give violet blue; logwood, red, changing to yellow.

The "brown phase" reaction¹ may be useful for the characterization of chlorophyll, when this has not been previously treated with alkalies. The green ether or petroleum ether solution of the coloring matter, when treated with a little methyl alcohol solution of potassium hydroxid, becomes brown, returning to green in a few moments.

The characteristic reaction of curcumin (turmeric) with boric acid may be conveniently carried out as follows: The aqueous or dilute alcoholic solution of the color is treated with hydrochloric acid until the shade just begins to appear slightly orange. The mixture is then divided into two parts and some boric acid powder or crystals added to one part. A marked reddening quickly will be apparent, best seen by comparison with the portion to which the boric acid has not been added.²

¹ Molisch, Ber. bot. Ges. 14 (1896), 16. Willstaetter and Stoll, Untersuchungen ueber Chlorophyll. Berlin, 1913, p. 144.

² The properties of pure preparations of the various natural coloring matters, as described by the numerous investigators who have isolated and studied them, are described for the most part in H. Rupe's *Chemie der Natürlichen Farbstoffe*, Braunschweig, 1900 and 1909. Properties of the chlorophylls and carotinoids are given by Willstaetter and Stoll, *Untersuchungen ueber Chlorophyll*, Berlin, 1913; those of the coloring matters of the cornflower, rose, pelargona flower, larkspur, cranberry, whortleberry, and purple grape, are described by Willstaetter and coworkers. Sitzb. kgl. Preuss. Akad. 12 (1914), 402, Liebigs Ann. d. Chem. 408 (1915) 1.

TABLE 7.—Numbers by which dyes are designated in different published tables.

[Under "G." numbers refer to *A Systematic Survey of the Organic Coloring Matters*, by A. G. Green, founded on the German of Drs. G. Schultz and P. Julius, London and New York, 1904; under "S." to *Farbstofftabellen*, by Dr. Gustav Schultz, Berlin, 1911-1914, under "M." to Mulliken's *A Method for the Identification of Pure Organic Compounds*, vol. 3, New York, 1910. One of the common names is also given.]

No.	Color.	G.	S.	M.	No.	Color.	G.	S.	M.
1	Acid Magenta.	462	524	245	64	Orange IV.	88	139	900
2	Light Green S F Bluish.	434	504		65	Azollavin.	92	140	910
3	Light Green S F Yellowish.	435	505	257	66	Fast Brown N.	101	160	849
4	Erioglaucin A.	436	506	287	67	Fast Red A.	102	161	777
5	Cyanol Extra.	439	546	285	68	Rosolic Acid.	483	555	249
6	Wool Green S.	491	566	279	69	Uranin.	510	585	1,141
7	Patent Blue.	440	543	277	70	Metachrome Orange R.	26	58	893
8	Nigrosin Soluble.	602	700	122	71	Chrysamin G.	220	342	1,061
9	Ponceau 6 R.	108	170	846	72	Chrysamin R.	269	394	1,060
10	Acid Yellow G.	8	137	918	73	Eosin.	512	587	1,129
11	Fast Yellow R.	9	149		74	Safrasin.	515	590	1,114
12	Brilliant Yellow S.	89	142		75	Erythrosin G.	516	591	
13	Indigo Carmine.	692	877	94	76	Erythrosin B.	517	592	1,113
14	Sun Yellow.	399	9	166	77	Phloxin.	518	593	1,107
15	New Coccin.	106	169	826	78	Rose Bengale.	520	595	1,109
16	Amaranth.	107	168	811	79	Eosin 10 B.	521	596	1,112
17	Tartrazin.	94	23	948	80	Rose Bengale 3 B.	523	597	1,103
18	Naphthol Green B.	398	4	951	81	Victoria Yellow.	2		
19	Azocarmine B.	605	673	75	82	Martius Yellow.	3	6	945
20	Azocarmine G.	604	672	71	83	Aurantin.	6		897
21	Naphthol Black B.	188	272	984	84	Alizarin.	534	778	905
22	Orange G.	21	41		85	Curcumin.	707	927	535
23	Fast Acid Fuchsin B.	21	41		86	Sudan G.	10	35	536
24	Chicago Blue 6 B.	318	424	575	87	Formyl Violet S 4 B.	468	530	307
25	Chromotrope 2 R.	20	40		88	Acid Violet N.	464	527	309
26	Azofuchsin G.	93	146	757	89	Night Green 2 B.	438	503	265
27	Soluble Blue.	480	539	212	90	Guinea Green B.	433	502	259
28	Palatine Scarlet.	53	81		91	Patent Blue A.	442	545	278
29	Ponceau 2 R.	55	82	834	92	Methyl Alkali Blue.	476	535	196
30	Fast Red E.	165	166		93	Congo Red.	240	307	412
31	Naphthol Yellow S.	4	7	946	94	Benzopurpurin 4 B.	277	363	1,020
32	Cochineal.	706	932	774	95	Alizarin Blue.	562	803	1,163
33	Ponceau 3 R.	56	83	833	96	Thioflavin T.	658	618	1,084
34	Palatine Red.	62	109		97	Rhodamin S.	496	570	143
35	Crystal Ponceau.	64	113	843	98	Methylene Blue.	650	659	23
36	Bordeaux B.	65	112	778	99	New Blue.	639	649	34
37	Azorubin.	103	163	783	100	Safranin.	584	679	8
38	Fast Brown.	139	213		101	Fuchsin.	448	512	139
39	Crocein Scarlet O extra.	164	251		102	Auramin O.	425	493	1,085
40	Quinolin Yellow water-soluble.	667	613		103	Auramin G.	426	494	
41	Crocein Scarlet 8 B.	169	255	802	104	Methyl Violet.	451	515	232
42	Biebrich Scarlet.	163	247	800	105	Crystal Violet.	452	516	229
43	Bordeaux G.	170	254	1,133	106	Malachite Green.	427	495	177
44	Resorcin Yellow.	84	143	830	107	Malachite Green G.	428	499	170
45	Brilliant Crocein M.	146	267		108	Bismarck Brown.	197	283	454
46	Azo Blue.	287	377	689	109	Bismarck Brown R.	201	284	1,030
47	Erika B.	78	121		110	Chrysoidin.	17	33	509
48	Azolitmin.	710	934		111	Chrysoidin R.	18	34	
49	Alizarin Red S.	546	780	1,111	112	Rhodamin 3 B.	505	574	1,002
50	Picric Acid.	1	5	947	113	Irisamin G.	499	576	1,012
51	Violamin R.	507	582	1,101	114	Rhodamin B.	504	573	1,001
52	Brilliant Yellow.	328	303		115	Rhodamin G.	502	572	1,101
53	Rosindulin 2 G.	606	674	78	116	Butter Yellow.	16	32	
54	Cloth Red B.	154	236	371	117	Anilin Yellow.	7	31	932
55	Orange I.	85	144	871	118	Yellow Fat Color.		68	
56	Crocein Orange.	13	37	877	121	Quinolin Yellow spirit soluble.	666	612	
57	Orange 2.	86	145	872	123	Sudan Brown.	59	105	
58	Orange R.	97	151		125	Sudan I.	11	36	887
59	Scarlet G R.	54			126	Sudan II.	49	76	1,026
60	Chrysophenin.	329	304	524	127	Carminaph Garnet.	60	106	
61	Resorcin Brown.	137	211	451	128	Sudan III.	143	223	
62	Bordeaux B X	157	237	1,132	129	Sudan IV.		232	839
63	Metanil Yellow.	95	134	901	130	Para Red.	31	56	